Roles of women in agriculture: A case study of rural Lahore, Pakistan

Wajiha Ishaq¹ and Shafique Qadir Memon²*

Key Message   Women of rural Lahore are actively involved in agriculture, mainly in post harvesting and livestock management practices. They are facing problems due to lack of education, resources and financial services.

ABSTRACT   In rural areas of Pakistan, the role of women in agriculture is more pronounced because they contribute a lot to agriculture, but their contribution in agriculture is not acknowledged. The present study was designed in rural union councils of three towns of Lahore, Pakistan namely Nishatar Town, Iqbal Town and Wahga Town. A questionnaire was designed to collect the data from rural women (n=207). Results reveals that a majority of women (42.02%) started the work at 29 to 39 years of age and mostly (86.95%) they were illiterate. A majority of the respondents (81.64%) were getting agricultural information from their own family members, and 56.52% rural women worked 8-10 hours per day. During this study, it was also found that wheat and rice were the major crops sown by all the respondents (100%). Out of all the pre-harvesting activities, 94.2% positive responses were recorded against seed bed preparation. A majority of rural women (85.02, 88.88 and 95.65%) were involved in shed cleaning, dung collection and fodder cutting, respectively. Thirty-one percent of rural women reported that limited agricultural product was the main cause for not being involved in agricultural marketing. Among the problems of household activities, 43.5% rural women reported that husband wife conflicts were the major problem in their life. Need assessment analysis reveals that rural women presented the highest need of trainings (85.5%) to improve their role in agricultural activities. This study suggests that concerted efforts of government and non-government agencies are required to uplift these rural women.

Keywords: Agriculture, Empowerment, Rural constraints, Role of women

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INTRODUCTION

Population is growing at a high speed in Pakistan, and a majority of the population is involved directly or indirectly with agriculture to generate their income (Butt et al., 2010). This sector is playing a key role in reducing poverty and acts as a source of growth in the countries where it is a main source of livelihood for the poor (Food and Agriculture Organization [FAO], 2011a; Cervantes-Godoy & Dewbre, 2010). It provides raw materials to industries and also serves as a market of its product thereby it contributes a lot to the national income (Begum & Yasmeen, 2011). It has several linkages with other non-farm rural activities and hence results in employment generation and income earning opportunities (Fatima, 2012). Both men and women play an important role in this sector. But in rural areas the role of women in agriculture is more pronounced and the most of the agricultural activities revolve around them (Begum & Yasmeen, 2011). In a previous research study by Luqman et al. (2006), it has been reported that women account 36.7% labour force of agriculture in the developed countries, while 43.6% labour force in underdeveloped countries. It has been estimated that more than fifty percent of the world food production has been done by women. Due to their vital role in the huge world food production, women are considered as the sources of knowledge for cultivating, processing as well as preserving of locally adapted nutritious crop varieties. Due to having such
type of knowledge, women may be recognized as the innovation leaders for sustainable development in agriculture (Chung, 2012).

The role of women in agricultural sector cannot be denied. They actively participate in the major field crops production and their intensity of participation is directly linked with their age, social class as well as the type of crop to be cultivated. The rural women work almost 12 to 15 hours a day. During sowing and harvesting of farming system, they look too busy to perform their duties honestly. For the production of wheat, cotton and vegetables, their participation is higher than that of other crops cultivation. A tremendous labour is required to perform various activities of cotton production and this type of labour is provided by the feminization in agriculture sector (FAO, 2015).

The women farmers work very hard. They perform a number of tasks, and remain busy from dawn to dusk (Nazir et al., 2013). Their activities typically include the production of agricultural crops, earning wages through agriculture and other rural business, participating in agricultural marketing as well as maintaining their homes. In economic point of view, most of these activities are not considered as active employment in national records but in actual figure, they are crucial for the welfare of rural households (FAO, 2011b). Batool et al. (2014) conducted a research study on the participatory role of women in dairy farm operations for smallholder system in Punjab-Pakistan and reported that women also take part in animal husbandry especially grazing, fodder cutting, feeding, transportation, milking, butter preparation and preservation, as well as cleanliness of livestock sheds. Besides these activities, women also play a crucial role in general health care, preparation of milk products and the marketing of these products (Ahmad, 2001).

Rural women contribute a lot to agriculture but their contribution in agriculture is not appreciated. Unfortunately, despite their wealth of knowledge and capability, they are ignored by policy makers, often not being acknowledged as “productive” farmers. Their farm work is often unpaid or under-valued and they tend to be debarred from decision-making (Ogunlela & Mukhtar, 2009). They are negatively affected by traditional pattern and economic policies (Amin et al., 2009a). They face more constraints than that of men. They have neither ownership nor control over resources. They are expected to give up their rights in favour of brothers or husbands (Pesticide Action Network UK, 2009). They are denied of their basic rights such as owning property, access to the health cares, getting education, securing bank credits and becoming a part of technology transfer. They are underprivileged to avail the opportunities of socio-economic development. They have lesser access to extension services, technology as well as trainings relative to their male counterparts (Nosheen et al., 2008).

The present study has been designed to highlight the enormous roles of the rural women in agriculture, determine the causes for women participation in agricultural activities, investigate different constraints faced and explore different factors which determine their empowerment. The study would help to bring hidden talents of rural women and would be valuable for the policy makers to formulate future policy guidelines for rural as well as agricultural development. We believe that no such type of study has been conducted in rural Lahore, Pakistan. So the findings of this study would constitute a significant addition to the existing literature.

**METHODOLOGY**

The study was conducted in rural union councils of three towns of Lahore namely Nishatar Town, Iqbal Town and Wahga Town. The reasons for selecting three towns were time and resources constraints. It was not possible for researcher to include all the rural union councils in given time and resources. At first stage, the simple random sampling technique was used to select nine rural union councils from these three towns through lottery method. At the second stage, the researcher made an effort to distribute 500 questionnaires. The reason for selecting a sample size of 500 for this study was made following (Sekaran and Bougie, 2010) who suggested that the sample size larger than 30 and less than 500 are appropriate for the most research works. The researcher collected the data herself at weekends and mostly in the absence of male households so that they could not influence the respondents. It was very difficult for researchers to collect data as the most of the respondents were illiterate, unable to understand the meaning and context of the questions. Therefore, face to face explanation of the questions was made possible by the researchers. It took three months to complete this extensive phase of data collection and 207 questionnaires were filled from all the selected rural union councils with return rate of 41.4%. The reasons for this low return rate lie behind the
religious values of our society, as well as cultural complexities in this kind of environment. Therefore, the contact with female household individually was very difficult and prohibited. Being a female researcher, I tried with my best to approach women but faced a lot of restrictions. At the last stage, the collected data was analyzed employing Statistical Package for Social Sciences (SPSS) software version 17.0 and both descriptive and inferential statistics were used to obtain the best results.

RESULTS

This study was conducted for the purpose of identifying the role of women in agriculture. For it, women role in rural areas of Lahore, Pakistan was interpreted for crop production and livestock management. Subsequently, the relation of these activities was assessed with household.

Selected characteristics of the rural women

The role of women in agriculture is considered as supporting seraphim in many societies of the world. The women start working in early age as compared to men. In present study, various demographic characteristics including age, educational status, sources of information, reasons for performing agricultural activities and working hours of the respondents were considered. The data regarding demographic characteristics of the respondents have been reported in table 1. Age is a key factor in adoption of an innovation and it has positive or negative impact on individual behavior (Siddiqui et al., 2003). Table 1 shows that a majority of women (42.02%) started the work at 29 to 39 years of age followed by the women (28.5%) who started the work at 18-28 years of age. The most of the women in the study area were illiterate (86.95%), while the educational status of 10.14% respondents was primary, 0.96% and 1.93% respondents were literate up to middle and matric level, respectively. No government or private organization was working in those areas for the rights of women farmers nor do they have information about such agencies. They were just working on their own as the major sources of occupational training and information. An overwhelming majority of the respondents (81.64%) were getting agricultural information from their own family members followed by the respondents (18.35%) that were securing agricultural information from their friends (Table 1). Table 1 also shows that a majority of women (63.76%) started this work to support their families economically. 20.77% of the respondents reported that they started agricultural activities due to family profession, and 11.59% women adapted this profession because no other work was available. However, only 3.9% of the respondents started the profession of agriculture because of personal interest. The data shows that a majority of the women were doing this kind of job only to support their families and they showed little interest in this profession. The study indicated that the most of the respondents were working for long hours in the fields. A majority of the women (56.52%) worked 8-10 hours per day followed by women (19.8 and 16.42%) who worked 11-13 hours and 5-7 hours a day, respectively. Only 7.24% women worked 2-4 hours per day. However, the average working hours of the respondents were calculated as 9 hours per day (Table 1). Hence, age of the respondents, education status, sources of information, reasons for performing the agricultural activities and working hours of the respondents show a mutual relationship with each other.

Crops sowing in study area

Table 2 reported the crops sown by the respondents in the research area and it is clear from the table that wheat and rice were the major crops sown by all the respondents (100%). It also shows that 74.87% respondents were cultivating vegetables in their fields followed by the respondents (84.54%) who were cultivating folder crops in their fields.

Role of rural women in agricultural activities

Women are dynamic worker not only in farms and fields but they also accomplish such activities as enhancements of agricultural productivity e.g. seed bed preparation, weeding, harvesting threshing, grain cleaning, food storage, cottage industry etc. Jamali (2009) reported that Pakistani rural women are not only involved in household accomplishments but also involved in rural socio-economic activities by adapting services of crop production, cotton industry and livestock management.
Table 3 describes the role of women in two different agricultural activities such as crop production and livestock management. However, out of all the pre-harvesting activities 94.2% positive responses were recorded against seed bed preparation and 47.82% against weeding. While all other activities were related to post-harvesting wherein all the 100% respondents showed positive responses for harvesting, 74.39% for picking of fruits, 74.39% for winnowing, 74.39% for drying and 100% for cleaning to transfer the agricultural produce to the market. Similarly, 85.02% women said that they were employing in making bundles and storing the harvested crops, respectively (Fig. 1). But all of the women said that threshing activities were performed by men.

The data presented in table 3 also shows the role of women in livestock management. It is clear from the table that the women did not perform the livestock activities such as grazing and bathing. A majority of rural women (85.02, 88.88 and 95.65%) were involved in shed cleaning, dung collection and fodder cutting, respectively. More than two-third (84.05%) of them were found to be involved in milking, yogurt preparation, and milk and yogurt storage, respectively. 79.71% women were seen to be involved in shed building. Regarding the ghee making activities of livestock management, 76.32% women were involved to prepare ghee. More than half women (52.65 and 67.63%) were performing the activities of dung cake making and providing the food and water to livestock, respectively (Fig. 1). Hence, the role of women in both crops and livestock activities was found to be higher than that of men in the study area.

Problems faced by rural women

Women contribution is measured to be lower in a country not because of their least possible involvement than that of men but because their involvement goes unacknowledged, unrecorded and unrecognized. In fact, they are mainly accountable for pre-cultivating and post-harvesting operations of food crops compulsory for household livelihood and their struggle goes unrecognized and unpaid (Government of Pakistan [GOP], 2014). Table 4 reports the problems faced by the rural women regarding agriculture marketing. There were four main causes recognized by rural women for problems in agricultural marketing (Table 4). Thirty-one percent of rural women reported that limited agricultural product is the main cause for not being involved in agricultural marketing. Thirty percent of rural women described that family opposition is the primary cause of limited role of women in agricultural marketing. Twenty-four percent of rural women mentioned that limited business and negotiation skills created problems that obstructed their participation in agricultural marketing. The problems faced by rural women in their household activities have also been reported in table 4. From the table, it is clear that 43.5% rural women reported that husband wife conflicts is the major problem in their life, while 36.6% mentioned the conflicts with in-laws. It was followed by the rural women (14.8%) who described that domestic violence is the major problem of households. The remaining 4.9% revealed that parents-children conflicts are the major problems in their life.

Need assessment of rural women

The need assessment highlights the suggestions of working women in order to improve their working and living conditions. The respondents were asked to select from a pre-prepared list of suggestions for their empowerment in agricultural activities to boost up their living standard. The suggestions were then graded according to the responses of the participants, and are presented in table 5. The table shows the needs of respondents in the form of percentages and the highest rankings are considered the most important need of the rural women. Analysis of need assessment reveals that rural women presented the highest need of trainings (85.5%) to improve their role in agricultural activities. While rural women play an essential role in agricultural production, thereby awareness should be created among rural women about the modern agricultural technologies which mean to educate them about these innovations. More or less 167 respondents (80%) expressed a need for awareness about modern agricultural technologies. This indicates that they have more space to increase the agricultural production by availing innovative information about agriculture. The main problem that rural women face in performing the agricultural activities is the lack of credit facilities. The microcredit programs should be arranged to promote the access of rural women to the micro credit and other financial services with subsidized loans or interest-free loans so that they actively participate in agricultural activities and may enhance the agricultural productivity. During this study, more than half of the
respondents (70%) expressed the need of micro credits and other financial services. The basic concept of market is any type of structure that permits the buyers and sellers to interchange any type of information, goods and services. The women are efficient decision makers and they show quick response to demands and supplies of the markets (Malik et al., 2015). A perusal of the table 5 reveals that more than half of the respondents (63.3%) considered that rural women should be given access to the markets. Information is an essential ingredient of integrated rural development that strengthens the link among various social groups to develop together as a well-organized society. The mass media such as TV, radio and newspapers is indispensable for disseminating information and educating the rural women (Lane, 2007). During this study, 59.4% respondents suggested that role of rural women should be highlighted through TV, radio and newspapers which will be helpful in the motivation of women to be actively participated in agricultural activities.

Table 1 Selected characteristics of the respondents

<table>
<thead>
<tr>
<th>Selected characteristics</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-28</td>
<td>59</td>
<td>28.50</td>
</tr>
<tr>
<td>29-39</td>
<td>87</td>
<td>42.02</td>
</tr>
<tr>
<td>40-50</td>
<td>46</td>
<td>22.22</td>
</tr>
<tr>
<td>51-61</td>
<td>15</td>
<td>7.24</td>
</tr>
<tr>
<td><strong>Educational status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>180</td>
<td>86.95</td>
</tr>
<tr>
<td>Primary</td>
<td>21</td>
<td>10.14</td>
</tr>
<tr>
<td>Middle</td>
<td>2</td>
<td>0.96</td>
</tr>
<tr>
<td>Matriculation</td>
<td>4</td>
<td>1.93</td>
</tr>
<tr>
<td><strong>Sources of information</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family</td>
<td>169</td>
<td>81.64</td>
</tr>
<tr>
<td>Friends</td>
<td>38</td>
<td>18.35</td>
</tr>
<tr>
<td><strong>Reasons for performing the agricultural activities</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family profession</td>
<td>43</td>
<td>20.77</td>
</tr>
<tr>
<td>Personal interest</td>
<td>8</td>
<td>3.86</td>
</tr>
<tr>
<td>Supporting the family economically</td>
<td>132</td>
<td>63.76</td>
</tr>
<tr>
<td>No availability of any other work</td>
<td>24</td>
<td>11.59</td>
</tr>
<tr>
<td><strong>Working hours of the respondents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-4</td>
<td>15</td>
<td>7.24</td>
</tr>
<tr>
<td>5-7</td>
<td>34</td>
<td>16.42</td>
</tr>
<tr>
<td>8-10</td>
<td>117</td>
<td>56.52</td>
</tr>
<tr>
<td>11-13</td>
<td>41</td>
<td>19.80</td>
</tr>
</tbody>
</table>

Table 2 Description of the crops sown by the respondents

<table>
<thead>
<tr>
<th>Crops</th>
<th>Frequency Yes</th>
<th>Percentage Yes</th>
<th>Frequency No</th>
<th>Percentage No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>207</td>
<td>100</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Rice</td>
<td>207</td>
<td>100</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Vegetables</td>
<td>155</td>
<td>74.87</td>
<td>52</td>
<td>25.12</td>
</tr>
<tr>
<td>Fodder</td>
<td>175</td>
<td>84.54</td>
<td>32</td>
<td>15.45</td>
</tr>
</tbody>
</table>
## Table 3: Description of the role of women in agricultural activities

<table>
<thead>
<tr>
<th>Activities</th>
<th>Frequency</th>
<th>%</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Crop production activities</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed bed preparation</td>
<td>195</td>
<td>94.2</td>
<td>12</td>
<td>5.79</td>
</tr>
<tr>
<td>Weeding</td>
<td>99</td>
<td>47.82</td>
<td>108</td>
<td>52.17</td>
</tr>
<tr>
<td>Harvesting</td>
<td>207</td>
<td>100</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Picking of fruits</td>
<td>154</td>
<td>74.39</td>
<td>53</td>
<td>25.6</td>
</tr>
<tr>
<td>Threshing</td>
<td>0</td>
<td>0.00</td>
<td>207</td>
<td>100</td>
</tr>
<tr>
<td>Winnowing</td>
<td>154</td>
<td>74.39</td>
<td>53</td>
<td>25.6</td>
</tr>
<tr>
<td>Drying</td>
<td>154</td>
<td>74.39</td>
<td>53</td>
<td>25.6</td>
</tr>
<tr>
<td>Cleaning</td>
<td>207</td>
<td>100</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Making bundles</td>
<td>176</td>
<td>85.02</td>
<td>31</td>
<td>14.97</td>
</tr>
<tr>
<td>Storing</td>
<td>176</td>
<td>85.02</td>
<td>31</td>
<td>14.97</td>
</tr>
<tr>
<td><strong>Livestock management activities</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fodder cutting</td>
<td>198</td>
<td>95.65</td>
<td>9</td>
<td>4.34</td>
</tr>
<tr>
<td>Feeding</td>
<td>140</td>
<td>67.63</td>
<td>67</td>
<td>32.36</td>
</tr>
<tr>
<td>Watering</td>
<td>140</td>
<td>67.63</td>
<td>67</td>
<td>32.36</td>
</tr>
<tr>
<td>Shed cleaning</td>
<td>176</td>
<td>85.02</td>
<td>31</td>
<td>14.97</td>
</tr>
<tr>
<td>Shed building</td>
<td>165</td>
<td>79.71</td>
<td>42</td>
<td>20.28</td>
</tr>
<tr>
<td>Grazing</td>
<td>0</td>
<td>0.00</td>
<td>207</td>
<td>100</td>
</tr>
<tr>
<td>Bathing</td>
<td>0</td>
<td>0.00</td>
<td>207</td>
<td>100</td>
</tr>
<tr>
<td>Dung collection</td>
<td>184</td>
<td>88.88</td>
<td>23</td>
<td>11.11</td>
</tr>
<tr>
<td>Dung cake making</td>
<td>109</td>
<td>52.65</td>
<td>98</td>
<td>47.34</td>
</tr>
<tr>
<td>Milking</td>
<td>174</td>
<td>84.05</td>
<td>33</td>
<td>15.94</td>
</tr>
<tr>
<td>Yogurt preparation</td>
<td>174</td>
<td>84.05</td>
<td>33</td>
<td>15.94</td>
</tr>
<tr>
<td>Ghee making</td>
<td>158</td>
<td>76.32</td>
<td>49</td>
<td>23.67</td>
</tr>
<tr>
<td>Milk and yogurt storage</td>
<td>174</td>
<td>84.05</td>
<td>33</td>
<td>15.94</td>
</tr>
</tbody>
</table>

## Table 4: Distribution of the respondents according to their problems faced in agricultural and household activities

<table>
<thead>
<tr>
<th>Problems faced by rural women</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In agricultural marketing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of transportation</td>
<td>24</td>
<td>24.5</td>
</tr>
<tr>
<td>Limited business and negotiation skills</td>
<td>13</td>
<td>13.3</td>
</tr>
<tr>
<td>Family opposition</td>
<td>30</td>
<td>30.6</td>
</tr>
<tr>
<td>Limited product</td>
<td>31</td>
<td>31.6</td>
</tr>
<tr>
<td><strong>In household activities</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Husband wife conflicts</td>
<td>44</td>
<td>43.5</td>
</tr>
<tr>
<td>Parent children conflicts</td>
<td>5</td>
<td>4.9</td>
</tr>
<tr>
<td>Conflicts with in-laws</td>
<td>37</td>
<td>36.6</td>
</tr>
<tr>
<td>Domestic violence</td>
<td>15</td>
<td>14.8</td>
</tr>
</tbody>
</table>
Table 5 Description of the suggestions for empowerment of the rural women

<table>
<thead>
<tr>
<th>Suggestions</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Role of rural women should be highlighted through TV, radio and newspapers</td>
<td>123</td>
<td>84</td>
</tr>
<tr>
<td>Awareness should be created among rural women about the modern agricultural technologies</td>
<td>167</td>
<td>84</td>
</tr>
<tr>
<td>Rural women should be given access to the markets</td>
<td>131</td>
<td>76</td>
</tr>
<tr>
<td>Micro credits and other financial services should be provided to the rural women</td>
<td>145</td>
<td>62</td>
</tr>
<tr>
<td>Trainings should be arranged for rural women</td>
<td>177</td>
<td>30</td>
</tr>
</tbody>
</table>

Fig. 1 Role of women in agricultural activities
DISCUSSION

Women carry out almost all the domestic work on the top priorities and their roles in outdoor productive activities are inevitable. This implies that women work very hard, but their work is invisible and unfortunately unpaid (Prakash, 2003). Our research study reveals that a majority of the respondents belonged to the low income groups and illiterate. They reported that they could not get education due to the lack of resources and even they were not able to send their children to schools due to poverty. In order to combat with the lack of resources/poverty, they were trying very hard. Our findings are supported by the findings of Nosheen et al. (2008) who conducted a study in Chakwal district, Pakistan to explore the gender role in decision making of agriculture. Their study reveals that 60.1% women were involved in management activities of livestock and they were also engaged with other agricultural activities including poultry husbandry, animal production and crop production and their share was 26.5%, 28% and 58.5% in poultry husbandry, animal production and crop production, respectively. They were in argument that 72% women were illiterate and their efforts were not appreciated in spite of putting equal efforts for farming activities.

Consistent to our findings, Iftikhar (2010) conducted a study on the involvement of rural women in agriculture sector in district Bahawalpur, Pakistan and found that though those rural women were having strong participation in farm and household activities yet majority of them (54%) were illiterate having little access to the basic infrastructure. A majority of the respondents i.e. 98% reported that they were receiving low income and they were facing a lot of problems due to want of technical experience, resources, technical guidance as well as agricultural information. They lost their life time opportunities of education, security and nutrition due to their diverse preoccupations. They are underdeveloped with respect to crop production and livestock management owing to lack of skills improving trainings causing low crop yields and inferior quality of the produce. Therefore, training and education in crop production, poultry production, fruit drying and livestock management should be arranged in spite of sociological constraints.

Our study found that women were playing a significant role in agriculture and were undertaking a number of activities in farming as well as in livestock such as sowing, weeding, harvesting, picking of fruits, drying, cleaning, winnowing, making bundles & storing and fodder cutting, feeding, watering, milking, building and cleaning sheds, dung collection, making dung cakes, yogurt & ghee making and storage. However, it was noticed that the women were mostly involved in post-harvesting, home activities and livestock management. These findings are in accordance with Hassan (2008) who carried out a study in district Muzaffargarh, Pakistan and concluded that both men and women were performing the activities of crop production except land preparation, fertilizer application, wheat threshing and fruit packing where only male respondents were actively involved. It was found that no woman was utilizing Extension Wing of Agriculture Department, private agencies and pesticide dealers as a source of information.

Congrous to our findings, Ali (2002) conducted a study on “women laborers in the fields of paddy” in tehsil Kamonki, district Gujranwala which revealed that important tasks like land preparation, water management, irrigation and fertilization were done by men, while women were involved in other activities of crop production. The work of males was more acknowledged than that of females by the family. In case of working hours, wages, decision making and availability of facilities, the females were facing discrimination. Our findings were also similar with that of Amin et al. (2009b) who attempted to recognize the women role regarding the post-harvest activities in the rural area of tehsil Faisalabad-Pakistan. They reported that post harvesting was done by rural women. The role of rural women was too important in performing various operations of post-harvesting such as drying, storing and cleaning of grains.

Our findings were in agreement with the previous research study by Khan et al. (2012) who assessed the role of women in agricultural activities in district Peshawar-Pakistan and found that women of the study area were actively participating in agricultural practices and spending a lot of time in performing post-harvesting operations. The major problems that women encountered during the operations of agricultural activities were the lack of extension services and trainings, financial and cultural constraints. Nazir et al. (2013) conducted a study in district Nankana Sahib and reported that rural women were actively participating in various agricultural activities such as sowing, harvesting and picking but facing a lot of problems during these activities.
Our findings indicate that most of the respondents were not involved in agricultural marketing. The reasons behind this fact include family opposition, limited business and negotiation skills, lack of transportation and limited product. It clearly indicated that the women of the study area were bound by cultural traditions and did not have the capacities and skills for marketing. These results are in accordance with the previous research study of Begum and Yasmine (2011); International Centre for Research on Women (2012). Our findings clearly demonstrated that the most of the respondents were working for long hours in the fields as well as they were managing their households performing a number of duties such as cooking, washing, mopping, child care, fuel fetching, shopping of household necessities. All this shows that they are losing their opportunity of getting education, nutrition, leisure time and security due to their diverse preoccupations. These findings are supported by the findings of Prakash (2003), Iftikhar (2010); FAO (2011b).

This study also shows that there were no training opportunities for the women, no credit facilities and moreover, their sources of information were only the families and friends. A similar type of study was conducted by Haq (2007) in district Multan-Pakistan and indicated that the literacy rate of cotton growers was 76%, while the literacy rate of cotton pickers (women) was only 27%. As far as information sources for cotton pickers are concerned, the most of them used their female’s friendships for gaining awareness about pesticides and related issues. They also benefited from neighborhoods and various relations for the same purpose. Neither any government or private organization was working in those areas for the rights of women farmers nor did they have any information about such agencies. Women are considered inferior to men as a large flock reported that they were paid less as compared to men, did not have the right to property/landownershight, did not have the right to decision making and even in most cases were not allowed to run the home budget. These results are in line with the results of FAO (2011b); Nkhonjera (2011).

Coherent with our findings, Afzal et al. (2009) attempted to identify the factors hampering women empowerment in decision making and extension work. Their study was confined to district Okara, Punjab, Pakistan and the results showed that a vast majority of women were illiterate, having small land farms and monthly family income less than Rs. 100,000 and living in joint family system. A large proportion of the respondents reported that they had to face problems and constraints in accessing agricultural extension services in the context of social, economic, cultural, and political issues and were having very low participation in decision making processes. Extension services were limited to men because of different reasons; first insufficient agricultural advice or the benefits, such as seeds and credit, second extension staff professionals were male, third our customs and norms of segregation, fourth domestic responsibilities and lack of mobility and ignorance of actual contribution of women. But they suggested that by improving financial and economic conditions of women they would become independent in their decisions, which can play a vital role to empower women.

Conclusively, our study focused on the participation of rural women in a variety of productive activities and we found that different problems were faced by them like illiteracy, time constraints, health problems, no access to extension services and gender discrimination. The present study has focused on the causes of women’s involvement in agriculture, domestic problems faced by them, analysis of their role in agricultural marketing and exploration of the factors which determine their empowerment.

**CONCLUSION AND RECOMMENDATIONS**

The study concluded that rural women of the study area had strong participation in activities such as crop production, livestock husbandry and home management but they have always been remained invisible. They were mostly involved in low grade activities, while their participation in land preparation, ploughing, manuring, irrigation, transplantation, water and pest management was zero. They were facing a lot of socio-economic and technological constraints like lack of facilities, unawareness about modern agricultural technologies and indifferent family attitude. No training opportunities and no micro credit facilities were available in those areas and women had no exposure about the existence of any organization working for their rights. They were not involved in agricultural marketing and they had negligible role in decision making. So the concerted efforts such as improvement of agricultural extension system, starting educational programs, establishing training centers and provision of credit facilities by government and non-government bodies are required for the uplift of these rural women.
Author Contribution Statement Shafique Qadir Memon generated the idea, supervised the research as well as edited the manuscript. Wajihah Ishaq conducted the research project and wrote the manuscript. Both the authors read and approved the manuscript to be published in Journal of Rural Development and Agriculture.

Conflict of Interest The authors declare that they have no conflict of interest.

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Farmers perception about yield losses of kinnow (*Citrus reticulate*) during its harvesting and post harvesting operations: A case study of tehsil Sargodha, Pakistan

Farhat Ullah Khan\(^1\)*, Nowshad Khan\(^1\) and Fouzia Anjum\(^1\)

**Key Message** During this research, various factors causing yield losses of kinnow during its harvesting and post-harvesting operations were studied in tehsil Sargodha Pakistan, which is famous for high production of kinnow.

**ABSTRACT** Kinnow is one of the major fruit crops of Pakistan and it is produced at a large scale in Punjab province of Pakistan. In Punjab province, the district Sargodha is famous for high production of kinnow. Unfortunately, during harvesting and post harvesting operations, the losses of kinnow yields in Pakistan are higher than that of other kinnow producing countries of the world. Therefore, this research study was conducted to observe various factors leading to yield losses of kinnow during harvesting and post harvesting. For it, 20 union councils of tehsil Sargodha were selected and from each union council, 10 kinnow growers were selected randomly. A total of 200 farmers were selected for the sample. The results revealed that 91% farmers reported fruit injuries during picking. A majority of farmers (67%) reported that 20% losses of the total yield occurred during picking, (51%) and 70% farmers did not have transport capabilities and storage facilities, respectively so losses occurred during these stages. Rough handling should be avoided during harvesting operation. Due to the defective marketing system, the farmers get low income which discourages the farmers from adopting recommended orchard management practices. Therefore, government should pay due attention to the establishment of an effective citrus marketing system.

**Keywords:** Harvest and post-harvest, Kinnow, Sargodha, Yield losses

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**INTRODUCTION**

Fruits are an important part of Pakistan's agricultural exports. The environment and soil of Pakistan is very favorable for the production of fruits. Due to the favorable environment approximately 30 types of fruits are commonly produced in Pakistan. The most common fruits produced in the country are citrus, mango, apple, dates, grapes, banana, melons and guava (Khan & Shaukat, 2006; Government of Pakistan, 2008-2009; Shahzad et al., 2015). In terms of area utilized, production and export, citrus are at the top among all other fruits produced in Pakistan (Ghafoor et al., 2008).

In Pakistan, it has been reported that 29.55% of the total area under fruit cultivation accounts for citrus, while 60% of total acreage under citrus cultivation is being used for kinnow cultivation (Government of Pakistan, 2003-2004). Likewise, due to more area under cultivation, the production and export of kinnow leads among the citrus fruits. In Pakistan, the highest citrus yielding province is Punjab that covers 95% of the total area of the country under citrus farming (Tahir, 2014). Pakistan is among the top ten kinnow producing countries. In Pakistan, citrus is playing a significant role in creating employment by engaging manpower in several activities ranging from its production to its harvesting. In an estimate, more than 75,000 people secure jobs by performing activities of kinnow production and its marketing in Pakistan (Sharif et al., 2005).
Pakistan exports kinnow to Kuwait, Saudi Arabia, Dubai, Bahrain, Oman, Qatar, Netherlands, Singapore, Indonesia, UK, Russia and Malaysia.

There are various harvesting and post-harvesting problems which citrus industries in Pakistan are facing. Due to these problems, the quality and quantity of the citrus fruits are being adversely affected. As a result, there is less export of the fruit that brings high economic loss to the country. In Pakistan, the common practice of mandarin harvesting is plucking of fruit along with pedicels and clusters of leaves that account for major injury during transportation.

The highest post-harvest loss of citrus especially kinnow has been reported in January to February. It is due to less usage of cold storage at domestic level of marketing, as severe cold prevails during these two months. The contractor tries to secure high price of fruit due to delayed plucking, but on the other hand, this delayed plucking highly affects the flowering of plants for the coming season. The producers like to prepare their orchards as early as possible. This situation produces clashes between these parties. Moreover, less use of cold storage also causes losses due to frost and diseases that cause significant financial loss for kinnow producers (Shah et al., 2015). Lack of knowledge about marketing results in pre-harvesting of citrus. The commission agents are not interested in transferring information about market price to producers and it is a great barrier for producers to participate actively in the process (Sharif et al., 2005).

The other major factors that contribute to post-harvest losses of citrus include operational efficiency, pre-cooling, treatments such as fungicides and waxes, and storage conditions (Kader & Arpaia, 2002). Ali (2004) investigated the marketing of citrus fruits in Pakistan and reported that traditional methods of citrus cultivation and non-technical harvesting were the core reasons affecting the production potential of citrus. In addition, these activities were also causing post-harvest losses. There is an immediate need to stabilize the market and guide farmers to adopt sustainable and effective harvesting methods. Keeping in view these facts, the present study was carried out to identify the causes of yield losses of kinnow (Mandarin) during harvesting and post harvesting (2011-2012). In this study, district Sargodha was selected as a study area because it is the main district of kinnow production in Pakistan.

METHODOLGY

The descriptive method of research was used for this study. Purposively, 20 union councils from tehsil Sargodha were selected, and 10 kinnow growers from each union council were taken on a random basis. (200) farmers were interviewed through interview schedule. The farmers were interviewed by a researcher personally at their homes and farms. Busy hours of the farmers were respected, and interviews were conducted at their free time. The objective of the research study was explained to the farmers before starting the actual interview. The data collected was tabulated systematically and analyzed statistically. Statistical Package for Social Sciences (SPSS) was used for data analysis. All the results were presented in counts and percentages in different tabular form. The associations of picking, storage, and post-harvest factors with yield of kinnow were tested with the help of chi-square test at 0.05 level of significance. Significance level is the probability of rejecting the null hypothesis when it is true. In this survey research, the data was analyzed at significance level of 0.05 which indicates a 5% risk of concluding that a difference exists when there is no actual difference.

RESULTS

The main objective of this research project was to study the yield losses of kinnow (Mandarin) during harvesting and post harvesting operations. Therefore farmers were asked various questions regarding the factors that cause kinnow losses.

Distribution of farmers for harvesting of kinnow fruits

The harvest season of citrus starts from September with harvesting of Feutral's Early and ends up to March with the harvesting of kinnow, of which sometimes the harvesting continues up to April. The appropriate time of harvest determines the net profit of the producers. The methods of harvesting of citrus are being changed from time to time and farmers tried to make improvements keeping in mind the resources and
technical knowledge. The farmers were asked about the method of picking of kinnow (Mandarin) fruit. The data in table 2 shows that all farmers were picking the fruit by hand with cutter.

**Losses of kinnow fruits during picking**

Farmers were asked about the fruit injury caused during picking of kinnow fruits. A majority of the farmers (91%) reported injuries of kinnow fruit during the picking. While 9% respondents answer was "No" about injuries of fruit during the picking, our study found a clear association of fruit injuries with yield of kinnow fruit (Table 3).

**Percentage of losses of kinnow fruit during picking**

The farmers were asked about percentage losses of kinnow fruit of the total yield during picking. The data presented in table 4 shows that a majority of the farmers (67%) reported that up to 20% losses of the total yield occurred during picking, followed by the farmers (20%) who reported losses between 21-35% during picking. Only 4% reported losses above 50% during picking, and 9% farmers reported no loss during picking. There was significance difference (P<0.05) between percent fruit losses and yield of kinnow.

**Distribution of farmers according to their access to kinnow market**

In Pakistan traditional marketing of agricultural products is not functioning efficiently in the modern market place. There are differences between prices paid by the consumers and prices received by the growers. In this way, marketing affects the production of kinnow fruits. Interviews were conducted to learn about the marketing problems and their effects on the yield of kinnow. The farmers were asked about the access to the kinnow market. The data presented in the table 5 shows that a large majority of the farmers (89%) had access to market. Only 11% farmers had no access to the market.

**Type of kinnow market/selling of kinnow fruit**

The farmers were asked about the type of kinnow market. The data given in table 6 shows that a majority of the farmers (68.5%) sold their fruit to the local agent.

**Ways of selling kinnow fruit**

Farmers were asked about their strategies for selling their fruit. A large number of the farmers (78%) sold their fruits through cash payment (Table 7). There was significance difference (P<0.05) between the way to sell kinnow fruit and the yield of the kinnow fruits. Late payment by the dealers and lower prices in the market discourage the growers, while reasonable prices and cash payments encourage the growers to adopt recommended harvesting and post harvesting technologies to get high citrus yield.

**Distribution of farmers with access to transport facilities**

The farmers were also asked about the transport facilities to carry fruit to the market. A majority of the farmers (51%) replied that they did not have transport facilities to carry fruit from the field to the market (Table 8). They hired transport to the market place.

**Fruit storage facilities**

Storage facilities for citrus are an important factor for stabilizing the price of citrus fruits. The farmers were also asked about the storage facilities of kinnow fruit. A majority of the farmers (70%) reported that they did not have storage facilities, while 30% farmers had storage facilities (Table 9). There was significance difference (P<0.05) between storage facilities and the yield of kinnow (Table 9). Proper storage facility is very important for marketing of kinnow. Non-availability of refrigerated transport facilities and poor condition of the roads, are responsible for high losses of kinnow. The farmers hire the cold storage at very high rate. Due
to the absence this storage facility, the yield of kinnow is adversely affected and thus loss to Pakistan Economy.

Preference to have fruit storage facilities

When farmers were asked if they would like to have better storage facilities, a majority of the respondents (67%) reported that this would benefit them. There was also significance difference (P<0.05) between the preference of the farmers to have storage facilities and the yield (Table 10).

Table 1 Provincial wise area and production of citrus cultivation in Pakistan

<table>
<thead>
<tr>
<th>Province</th>
<th>Area (Hectares)</th>
<th>Production (Tones)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Punjab</td>
<td>182558</td>
<td>2328090</td>
</tr>
<tr>
<td>Sindh</td>
<td>4930</td>
<td>29668</td>
</tr>
<tr>
<td>Khyber Pakhtunkhwa</td>
<td>3840</td>
<td>30871</td>
</tr>
<tr>
<td>Baluchistan</td>
<td>1504</td>
<td>6921</td>
</tr>
</tbody>
</table>

Source: Fruit, Vegetables and Condiments Statistics of Pakistan, 2014-15

Table 2 Distribution of farmers for harvesting of kinnow fruits

<table>
<thead>
<tr>
<th>Fruit picking</th>
<th>Frequency</th>
<th>Percentage</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual</td>
<td>200</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>Any other method</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3 Losses of kinnow fruits during picking

<table>
<thead>
<tr>
<th>Response</th>
<th>Frequency</th>
<th>Percentage</th>
<th>Chi-square significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>182</td>
<td>91.0</td>
<td>0.001</td>
</tr>
<tr>
<td>No</td>
<td>18</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Table 4 Percentage of losses of kinnow fruit during picking

<table>
<thead>
<tr>
<th>Response</th>
<th>Frequency</th>
<th>Percentage</th>
<th>Chi-square significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>No losses</td>
<td>18</td>
<td>9.0</td>
<td>0.000</td>
</tr>
<tr>
<td>Up to 20%</td>
<td>134</td>
<td>67.0</td>
<td></td>
</tr>
<tr>
<td>21-35%</td>
<td>40</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>36-50%</td>
<td>8</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>Above 50%</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Table 5 Distribution of farmers with access to kinnow market

<table>
<thead>
<tr>
<th>Response</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>178</td>
<td>89</td>
</tr>
<tr>
<td>No</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>100</td>
</tr>
</tbody>
</table>
### Table 6 Type of kinnow market/selling of kinnow fruit

<table>
<thead>
<tr>
<th>Response</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>No access to market</td>
<td>22</td>
<td>11.0</td>
</tr>
<tr>
<td>Local agent</td>
<td>137</td>
<td>68.5</td>
</tr>
<tr>
<td>Wholesale</td>
<td>31</td>
<td>15.5</td>
</tr>
<tr>
<td>Personal order</td>
<td>8</td>
<td>4.0</td>
</tr>
<tr>
<td>Any other</td>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 7 Ways of selling kinnow fruit

<table>
<thead>
<tr>
<th>Response</th>
<th>Frequency</th>
<th>Percentage</th>
<th>Chi-square significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cash payment</td>
<td>156</td>
<td>78.0</td>
<td>0.000</td>
</tr>
<tr>
<td>Advance payment</td>
<td>32</td>
<td>16.0</td>
<td></td>
</tr>
<tr>
<td>Loan</td>
<td>8 (4.0)</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>Any other</td>
<td>4 (2.0)</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>200 (100.0)</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

### Table 8 Distribution of farmers with access to transport facilities

<table>
<thead>
<tr>
<th>Response</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>98</td>
<td>49.0</td>
</tr>
<tr>
<td>No</td>
<td>102</td>
<td>51.0</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 9 Fruit storage facilities

<table>
<thead>
<tr>
<th>Response</th>
<th>Frequency</th>
<th>Percentage</th>
<th>Chi-square significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>60</td>
<td>30.0</td>
<td>0.000</td>
</tr>
<tr>
<td>No</td>
<td>140</td>
<td>70.0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

### Table 10 Preference to have fruit storage facilities

<table>
<thead>
<tr>
<th>Response</th>
<th>Frequency</th>
<th>Percentage</th>
<th>Chi-square significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Had facility</td>
<td>60</td>
<td>30.0</td>
<td>0.002</td>
</tr>
<tr>
<td>Yes</td>
<td>134</td>
<td>67.0</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>6</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

Pakistan is among the top ten kinnow producing countries. Kinnow is produced at a large scale in province Punjab, Pakistan. In addition to providing income, it also generates employment for the poor masses involving them in diverse activities from production to marketing. But its yield is reduced drastically during harvesting and post harvesting operations. The highest loss of kinnow production occurs in January-February due to unavailability of cold storage at domestic level resulting in severe diseases that cause significant financial loss for kinnow growers (Shah et al., 2015). We were interested to learn about the farmers perceptions about the yield losses of kinnow during harvesting and post harvesting activities. Therefore, a descriptive method of study was employed in tehsil Sargodha because in Punjab province, Sargodha is at the top in kinnow production.

Coherent with our findings, Ghafoor et al. (2010) conducted a study to investigate harvesting and marketing problems faced by citrus growers. They reported that lack of storage facility and non-availability of packaging materials like carton were the major constraints of harvesting and post harvesting of citrus being faced by the farmers. In addition, farmers were of the view that late payment by dealers, low price of kinnow in domestic market, middleman monopoly, high handling charges, inadequate storage facility, packaging and loading factors were hampering the productivity potential of citrus. Our findings were in agreement with the earlier research report by Ahmed et al. (2015) who conducted a study to quantify the post-harvest losses of kinnow at various stages i.e. at farm, wholesale market and retail levels of district Sargodha Pakistan. They reported that post-harvest losses at farm, wholesale market and retail levels were 72, 25 and 3% of the total post-harvest losses of kinnow, respectively. Overall post-harvest losses (45%) of the total production were reported in the study area. Their study suggested a scientific approach to minimize losses and this empirical study estimated the major determinants of post-harvest losses at transportation and retail level, as well as farm level.

Vadivelu and Kiran (2013) defined agricultural marketing as commercial functions which are involved in transporting agricultural products from producer to user. Another dimension reflected by agricultural marketing is supplying the products from rural areas to rural, urban and industrial areas. In modern era with the introduction of latest technologies and agricultural revolution, many stakeholders like farmers, middlemen, commission agents and customers are involved which have completely re-shaped the process. Various activities are involved in the whole process from planning production to the sale. It covers the phases of growing, harvesting, grading, packaging, transporting, storing, food processing, distributing and advertising. Hence agricultural marketing is a complete and vast process that requires a complete system of exchanging information. Anjum (2000) reported that there are two types of marketing systems in Pakistan. One is primitive marketing system and the other is traditional marketing system with little degree of scientific approach. During the study, it was found that the marketing structure was diversified and flexible in nature depending upon commodity’s problems and conditions prevailing in various parts of the country. Market is still controlled by merchants, village traders and commission agents and there is not improvement in the marketing system. The physical infrastructure in the markets like cold storage, adequate transport arrangements and processing plants are also inadequate.

Our findings coincided with the earlier proposition by Farooq et al. (2016) who studied the problems of fruit growers to their access to market in district Neelum Azad Jammu and Kashmir. It was an imperative phase to see the lives of masses, while spending a reasonable time period of life in the plantation of fruit trees and expecting the margins in the long run. The fruit production in Pakistan is expanded and more than 21 varieties of fruits are being produced in the country. The study drew attention to the problems faced by fruit growers towards the market access. The study concluded that fruit growers faced numerous problems in the process of fruit growth. The rudimentary apathy evaluated so far is the absence of basic civic facilities i.e. awareness, sprays, fertilizers, market related issues and road access which might not be difficult task for the government to access such fertile area and support people for the revenue generation activity. However, there is a need of special attention i.e. provision of pesticides, protection of fruit trees and provision of new varieties of fruit trees and adequate irrigation facilities along with the packing and pricing factors which impede the small scale fruit growers specifically and large scale fruit growers generally to access market and restrict the fruit growers to produce a reasonable revenue for their livelihood. A similar nature of study was conducted by Basra and Farooq (2006) who revealed that there are many marketing problems in Pakistan.
such as monopoly of middlemen, false weighing, illegal dues and deductions, involvement of local contractors, lack of marketing extension service and lack of advance marketing loans to small farmers. Abdullah and Hossain (2013) revealed that markets are located at urban areas so the farmers usually try to sell the produce at farm gate to avoid the transportation and other costs. As a result, they have to depend upon the commission agents who make payments to farmers at the spot or make promise to pay money within a stated time period. These middlemen pay lower price as compared to the market price.

CONCLUSION

All the farmers were picking the fruit by hand with cutter. The most of the farmers (91%) reported injuries of the fruit during picking. A majority of farmers (67%) reported 20% losses of the total yield during picking. There was close association among fruit injury, fruit losses and the yield. (89%) of the farmers had access to kinnow (Mandarin) market. (68.5%) of the farmers sold their fruit to the local agent. 78% farmers sold their fruits by cash payment. A majority of the farmers (51%) did not have transport facilities. A majority of farmers (70%) did not have storage facilities. 67% of farmers wanted to have storage facilities.

RECOMMENDATIONS

1. The kinnow picker must be trained to avoid any loss or injury of fruit during picking. Rough handling should be avoided during harvesting operation. The fruit should not be pulled from the branches during harvesting as the skin from the stem end can be ruptured. Long stems left on the fruit must be removed during packing, as the stem left on the fruit can damage other fruits, thereby causing spoilage and fruit loss. Ladders should be used to reach fruits born on high branches. Ladder placement in the tree should do carefully to avoid damaging fruit on the tree and limb breakage.
2. Due to the defective marketing system, the farmers get low income which discourages the farmers to adopt recommended orchard management practices. Therefore, government should pay due attention to the establishment of effective citrus marketing system.
3. Cold storage facilities should be made available in the vicinity of kinnow growing areas. Due to the lack of proper warehouses to store kinnow and existing storage facilities, all the fruits and vegetables are being kept in a similar temperature under one roof, which reduces the shelf life of the fruits.

Author Contribution Statement Nowshad Khan generated the idea and supervised the research. Farhat Ullah Khan conducted the research project and analyzed the data. Fouzia Anjum helped in manuscript writing. All the authors read and approved the manuscript.

Conflict of Interest The authors declare that they have no conflict of interest.

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Organic farming: Hope for the sustainable livelihoods of future generations in Pakistan

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ABSTRACT Organic farming is centuries old human and natural intervention which has been eco-friendly and economically viable. It comprises eco-friendly agricultural techniques leading towards the maximum production without using chemical inputs. Nature friendly farming approach helps in reducing the negative effects of environmental pollution by recycling crop rotation, using crop debris, farm yard manure, pest control with biological methods, appropriate tillage, cultivating legumes to add organic matter in the soil and to mitigate the climate hazards. A majority of rural population in Pakistan is willing to adapt the organic farming practices to save their input costs for better livelihoods. The present component of Pakistan economy is agriculture which contributes 21% to GDP and adds more than 45% labor in agricultural activities. The Pakistani farm-home women are playing a great role in agricultural development and livestock management. Organic farming is efficient for eco-system providing a balance in the life of human, crops and animals; hence leading to the sustainability of the system.

Keywords: Agricultural development, Food security, Future prospects, Livelihoods, Organic farming

INTRODUCTION

Organic farming movement started in many countries in response to the dangers to the livelihoods of the farming community and their surroundings (Rundgren, 2006). Now-a-days, 85% of the world produce is contributed by smallholders that supply food to the needy population. A majority of the smallholders face poverty and cannot afford costly inputs (International Federation of Organic Agriculture Movements [IFOAM], 2015).

Due to increase of contaminants in food items contributed by chemical pesticides and fertilizers; health risks are increasing at a high level. These chemicals also have bad effect on wildlife as well as other beneficial organisms like earth worms which help in improving the soil health (Maass Wolfenson, 2013). Organic farming is the solution to recover the soil made of salt intake by local natural organic matter as a ground cover with compost and organic fertilizer (Yousafzai et al., 2016). The source of the production of compost and organic fertilizer which contributes much to the economy of Pakistan is livestock. It plays a crucial role to support family income and jobs for the landless, smallholders and women in rural communities. It has been estimated that 30-35 million rural populations of Pakistan is getting 30-40% of their income through livestock directly or indirectly (Government of Pakistan [GOP], 2007a). Organic food fulfills the human nutritional vitamins, minerals, enzymes and micronutrients. Pakistan has to produce extra food grains to meet the dietary needs of the increasing population which is currently over 191.71 million (Pakistan Economic Survey, 2014-15). Overall dietary needs of the population have reached to twenty million tons wheat (Alam, 2003). It has been reported that harmful pesticides spray applied by the farming community contaminated nearly 10-15 percent of stored food during storage (Ali et al., 2011). Moreover, the researchers face the challenges of post-harvest losses. During sixties, Green Revolution was introduced in developing
countries through the use of chemical inputs and high yielding crop seeds. This increased food production but caused health and environmental risks (Ahmed et al., 2002). The similar scenario was witnessed in the entire world where gains were achieved putting life into risks, so an urgent response was needed to combat the alarming situation (Food and Agriculture Organization [FAO], 2012).

Conventional agriculture produces about one third of global greenhouse gases by the use of chemical inputs, machinery and livestock (FAO, 2011a). It results in wind and water erosion from soil surface, loss of soil fertility, water holding capacity and desertification due to overgrazing especially in Africa and northern meadows in Pakistan (FAO, 2011b). Moreover, pesticides and herbicides are accumulated in groundwater below the agricultural lands. Other pest and disease problems include more than four hundred pests and seventy dangerous pathogens have become resistant to one or more pesticides (Labelling Ecology Approved Fabrics [LEAF], 2010). Salinity and sodicity affected nearly 6.28 million hectares land in Pakistan and caused potential yield reduction. Soil degradation through natural hazards has also damaged the soil. The maximum erosion rate estimated was 150-165 tones/hectare/year (Bhutta, 2010).

Organic farming is the solution to recover the soil made of salt intake by local natural organic matter as a ground cover with compost and organic fertilizer (Baldwin, 2006). The source of the production of compost and organic fertilizer which contributes much to the economy of Pakistan is livestock (Chaudhry et al., 1999). It is estimated that 30-35 million rural populations of Pakistan is getting 30-40 percent of their income through livestock directly or indirectly (GOP, 2006). The fertilizer gap can be filled by organic farming with the addition of bio-fertilizers, vermin-composting, composting as well as crop rotation of cover and leguminous crops (Ali et al., 2015).

During the last few years, the horrific events such as climate change, natural disasters, land degradation, pollution of the environment and decline of biodiversity posed a threat to food security and nutrition in the world and created a state of development challenges related to the environment, socio-economic and security (United Nations International Strategy for Disaster Reduction [UNISDR], 2012). The safe food production threatens the most vulnerable people of the world, and high food prices have reversed the achievements in decreasing hunger as well as poverty. A serious and united response is needed among different people of the world to combat the problem of safe food production (FAO, 2008). Narayanan (2005) reported that conventional agriculture is unsustainable due to reduction of crop production, damage of environment and contamination caused by chemicals. An alternative method of crop production has emerged in the form of organic farming that produces good quality foods by using ecological phenomenon. Willer and Kilcher (2011) were in argument that organic farming can be performed by composting, cover cropping, intercropping, green manuring, farm yard manuring, effective microorganisms technology, mulching, and use of humic acids. Organic farming helps the communities to take interest in community development and safe environment (Wyatt, 2010).

Organic farming is a method which deals with the growing of plants and rearing of livestock in a natural way. This method involves the use of local biological material by avoiding chemical substances to maintain and enhance soil fertility and environmental balance by minimizing the pollution and wastage of resources. It involves the nature friendly agricultural principles like green manure, organic waste, integrated pest management (IPM) and crop rotation. In organic farming, little use of pesticides as well as fertilizers becomes possible only if these are natural and do not give any harm to the environment (Kesavan & Swaminathan, 2008).

When organic wastes (plant debris, farm yard manure, grass clipping, decomposed leaves etc) is compiled, the decomposition process starts. The microorganisms begin to decompose the organic materials and the temperature inside the pile rises up to 54-65 °C within three to four weeks of decomposing. It is very important to maintain the adequate aeration and moisture during the microbial activity that produces the compost in the shortest period of time. When the organic matter is completely decomposed and the temperature decreases to about 37 °C, then the compost is ready for use. On-farm composting method includes piles, pit composting and vessel composting (Morgera et al., 2012).
Prospects of eco-friendly agriculture in Pakistan

1. Pakistan and organic agriculture

The current agriculture system is an essential component of Pakistan’s economy and it accounts 21% share to gross domestic product. It acts as a source of employment as human labor for more than 45% people of Pakistan, while 60% of rural population is directly involved in this sector for its livelihoods (GOP, 2007b). Pakistan has a great potential for organic farming as the most of the farming community is interested in adapting organic farming technologies for their sustainable livelihoods and biodiversity in addition to reducing their farm expenses (Husnain & Khan, 2015). There is a need of awareness among the people of Pakistan about the benefits of organic farming. Eco-farming is being practiced in Pakistan at about six million hectares of the cultivable land is arid and rainfed regions (Baig et al., 2013). The soils in Baluchistan, Azad Jammu and Kashmir (AJK), Gilgit-Baltistan, Khyber Pakhtunkhwa (KPK) and northern areas of Punjab are available for organic farming where organic fertilizers and natural pest control with favorable climatic conditions are only tools for the farming community (Rasul & Hussain, 2015). During 2008, National Institute of Organic Agriculture (NIOA) was established at National Agricultural Research Centre, Islamabad. This institute is involved in working on organic farming techniques and disseminating new knowledge to the smallholders across the country (Musa et al., 2015).

Unfortunately, there is lack of inspection and certification system of organic products due to which their export is limiting to a great extent. The agriculture income is decreasing due to poor price policies of the government related to markets of agriculture products. Therefore, it is crucial to transfer the latest technologies of organic agriculture to the farming communities and to allow the rural women to participate in organic production. Easy loans and regular trainings should be arranged for these women to improve the organic production. The sustainable agriculture develops the farmer capacity especially stallholder and farm-home women to achieve the goal of their own resources like water, soil, meadows and forests. It also creates the farmers capacity to use their own local natural resources for the growth of their living standards which ultimately contribute in economic growth of the country (FAO, 2014).

The smallholder eco-friendly agriculture is the key to open the window towards sustainable future by saving the millions of souls from hunger and malnutrition (Serageldin & Steeds, 1997). There is an alternate option in the shape of home gardening trainings, bio-control of harmful insects/pests, Farmer Field Schools (FFS), nursery raising, seed bank, strategies of rain harvesting, conservation of land and water, and provision of poultry and livestock to the needy peasants. The Pakistani farm-home women play a great role in agricultural development and livestock management. The women farmers produce 80% of food and have a close relationship with land and food production of Pakistan (FAO, 2011d).

2. Plant protection and low yield

Although the gap between extension and research is very broad but it can be filled with mass education. In Pakistani rural society, the small medium farmers comprise 93% of the whole farming community, of which 81% cultivate less than 12.5 acres of land (World Bank, 2007). This majority of smallholders always face problems in seeking the field solutions from agricultural extension workers. Research and extension are the two basic elements of agricultural development. At present, the linkage between these two components is very weak. This gap can be filled with adequate funds, proper capacity building trainings, coordination between research, extension and the farming community. Research organizations should establish outreach components to design and evaluate appropriate extension methodologies by using information and communication technology (Rwelamira, 2015). The average of major crops production in Pakistan is around 50-83% which is less than that of the developed countries (Iqbal & Ahmed, 2005). The realization of this potential could offer unfinished tremendous opportunity for the future growth of agriculture. The introduction of valuable crops such as saffron, herbs, mushrooms and dried fruits in specific areas can bring a revolution in the lives of people. This revolution can occur only in a few years after the establishment of local cooperative system as well as the export facility supported by the organic certification system.

Organic farming can play a vital role in the betterment of rural and urban communities. Two thematic evaluations regarding organic agriculture and poverty reduction were done by International Fund for
Agricultural Development (IFAD) during 2001-2004 in Latin America, Caribbean and Asia (China and India). The evaluations examined the practices of organic methods associated with poverty reduction, food security and trade. The results were very promising for adapting nature friendly agriculture techniques along with better marketing (International Fund for Agricultural Development [IFAD], 2013).

As far as the use of chemical fertilizers is concerned; its use can be minimized by the preparation of organic fertilizers on the farm which is quite simple. Different organic substrates are easy to find in rural areas such as plant debris, leaves, grasses and weeds, farmyard manure, phosphate rock, press mud, gypsum, sulphur mud and green algae etc. (Kadir et al., 2016). The material is decomposed after mixing the ingredients and covered with plastic sheet which usually takes 45 days. This simple activity can save a huge number of the farming community. Organic fertilizers give the excellent results when used in crop rotations and green manure. The soil health can be restored every 4-5 years with the cultivation of leguminous crops to add organic matter and maintain the health of the soil (National Institute of Organic Agriculture [NIOA], 2012).

Local poisonous plants like neem (Azadharacta indica), Aak (Calotropis procera), Arosa (Adhatoda vasica), chili (Capsicum annum), garlic (Allium sativum) with insecticidal properties contribute well in the management of harmful pests. The bio-pesticides have remarkable effect against the leaf cutters along with repelling the sucking insects (Directorate of Organic Farming [DOF], 2010).

3. Conservation agriculture

Conservation agriculture reduces the requirement of water up to 70%. The countries of Latin American are predominantly adapting the soil conservation techniques at 10% of the total world area (FAO, 2010). Only 1% increase in farm area has been recorded in the last 52 years in Pakistan, which was originally 48.6% at that time. The 18% area of Pakistan is rainy, while nearly 82% of the farm land is irrigated. With the help of water harvesting techniques nearly 0.9 million hectares can be brought under irrigation for better production. Organic agriculture and better drainage facilities can save more than 21.5 billion rupees per year. If the rainy water collecting tanks may be developed to drive the irrigation water, then a large area of cultivable wasteland (about nine million hectares) can provide good agricultural production. Moreover, a huge amount of money (about 21.5 billion rupees) can be protected per year if the soils are improved by developing drainage system as well as the use of organic material (South Asian Association for Regional Cooperation [SAARC], 2011).

4. Condition of marketing system

There are four climates in Pakistan. Dry hot areas of Pakistan produce fruits like dates, citrus, mangoes, while other three cold climatic zones produce apricots, apples and plums etc in Gilgit-Baltistan, Quetta and Swat valley. The most of the fruit grown in Gilgit-Baltistan ripen at the end of summer and the start of rainy season (Gilgit Baltistan Bulletin, 2011). Evidently, this is not the ideal climate for sun drying of fruits. Even when the day is sunny, the night temperature falls rapidly resulting in dew and frost. Any fruit left out in the open area is thus damaged. Moreover, a huge loss of fruit occurs due to lack of enough roads from farmer field to the market, improper post-harvesting techniques, non-availability of skilled labor, lack of easy loans as well as serious diseases of fruit. Due to these problems, 30-50 percent of the fruit production is destroyed (Khan, 2012). The valleys of Baltistan, Gilgit and Hunza are producing apricot, mulberry, almond, apricot, cherry and apple. In these areas, each village is producing dry apricot (8-10 tons), dry mulberry (1-1.5 tons), almond (1 ton), dry apricot (5 tons), fresh cherry (2 tons) and fresh apple (25 tons) (“Hunza Apricots: Reaching Great Heights,” 2006). Large quantities of fresh and dry fruit are being produced from northern regions of Pakistan but due to lack of necessary management practices, more than half of the quantity is being wasted (Alam & Mujtaba, 2002).

The local producers in northern areas are facing many challenges in fruit harvesting, processing, grading, marketing and trading with foreign states to earn sufficient income for enhancing the living standards of the local people. Moreover, the scope of sea buckthorn and wild mushroom has potential in these regions to earn billions of rupees every year. Sea buckthorn is abundantly available in northern areas where a heavy capital can be earned and employment can be offered to the local people (Niaz, 2009).
The northern and southern areas of Kashmir having blessed lands produce cherries, pear, apples, walnut, citrus, mangoes and guava ("Economic Survey 2014-15: A General Review," 2015). Billions of dollars can be earned annually by providing marketing facilities in association with local agriculture related organizations having organic certification facility. The same areas in Baluchistan, Sindh and desert regions must be given priority in training facilities, storages, small equipments like solar driers, bio-gas units, post-harvest technology, packing, labeling and transportation.

Private trading agencies can contribute by cooperating with the public bodies to establish a strong marketing system to fulfill the local and foreign needs. A plan to support small scale agro-based industries in rural areas would check the migration of youth towards cities and thus the burden on cities will be reduced in terms of shortage of resources.

5. Development of small farms

The most of the rural society in Pakistanis lacking agricultural skills and the resources are destroyed such as burning of wheat straw after harvest, injudicious use of field grasses and plant debris, the post-harvest losses and huge grain losses due to inadequate storage facilities (Siddiqui & Sarwar, 2002). In many parts of Pakistan, warm climate is too favorable for the cultivation of flowers and herbs with a huge export potential in other countries. Value addition is another activity to earn huge capital, and the circumstances are still favorable if only a small support is made possible by the government to the smallholders. Farm-house women can bring a revolution in the community by providing organic food on the cost of small and simple loans in Pakistan.

6. Plant breeding techniques and issues of certified seeds

Agricultural research and development (R&D) mentions the most fruitful investment on agriculture sector in developing countries. R&D also refers to the investment on education, infrastructure and input credits. During 1965-2000, almost 50% of global crop production was achieved due to better crop management and improved plant varieties (Martino & Baethgen, 2014). The plant breeding division of agriculture has been severely affected due to the reason that various plant genetic organizations of Pakistan have not invested to boost up this division. The high yielding varieties can be developed by the conservation and sustainable use of plant genetic resources of plant breeding. These varieties would be efficient in utilizing nutrients and water as well as adaptable under biotic and abiotic stress conditions (Haggag et al., 2015). In Pakistan, during the past, the activity of private seed companies has increased due to the liberal government. Priorities should be given to private farms near agricultural research stations for seed production. In addition, seed certification services must strengthen their role to ensure food standards. A struggle is being done by Monsanto and other multinational organizations of seeds to seize and control over the global seed market and to make the farmers slaves. This is indeed an alarming situation for the developing world and its solution is only to save our own indigenous seeds that would be adaptable in our environment.

7. Water harvesting techniques

The benefits of irrigation are very important however judicious use of water plays a very important role in crop production and sustainable agro eco-system. It is estimated that irrigated agriculture in poor countries with 20% of all cultivable land is used for 47% of all crop production (FAO, 2016). The use of the best irrigation techniques contributes in feeding 9 billion people with expansion of irrigated areas (Foley, 2014). Pender (2008) reported that water harvesting and soil moisture conservation techniques are crucial to improve the fertility of soil and to reduce costs of chemical fertilizers and pesticides. The water courses can be channelized on water reservoirs and in deserts during the rainy season to save from the drought-hits, in addition to check dams that are built to save water for future requirements. Many beneficial traditional management techniques can help to expand the cropping area even in deserts or in changing climates. Today, the storage capacity of water has been limited to 30 days of supply, while the recommended storage capacity is 1000 days for countries having similar climate (Khan, 2014). Increase in temperature affects the snowmelt and flow of the Indus River, the main power source.
8. Urban agriculture

The population in the cities of Pakistan is increasing due to migration from rural villages putting enormous economic pressure and creating environmental pollution and health hazards (Azam & Khan, 2015). Roof top gardens have become popular among residents of large cities by creating greenhouses on the rooftops. Organically produced food in this method is safe, economical and less resources are needed (Germain et al., 2008). Moreover, the empty places on the edges of roads, canals and parks can be taken to grow for legumes, leafy vegetables and fruit trees. Herbal, vegetable and flower gardens can produce organic food as well as employment opportunities for the poor masses of the cities. At the same time, environmental pollution can be reduced and the fresh air is available for good health. The city waste can be turned in to compost and huge capital can be earned by this activity with the cooperation of private-public partnership (Modak et al., 2010).

9. Ecologically-based organic model in Brazil

The progress in eco-friendly agriculture in Brazil was initiated by rural elite agriculturists and agriculture-based professionals (Brandenburg, 2002). Ecologically-centered farming system was promoted by strong political system that is involved with the progress of a new model of society, associated with equality and justice. More than 55% of the Brazilian population is a part of the middle class. It has been noticed that in Brazil 70% of all the food consumed comes from the small holders. The great success of Brazil is multiplying the food quantity without expanding the use of lands and damaging the environment. This is also a tool against climate change and ecological imbalance. The main tools of agricultural production in Brazil are elimination of subsidies, enough funding and resources allocated for R&D and agro-based industry in rural areas (Madre & Devuyst, 2016). Moreover, strengthening of contact farming, promotion of family entrepreneurs, creation of domestic markets and cooperatives are playing a vital role. Small holding farmers were promoted in the area of direct sale-system and market safety. The modernization of agriculture in Brazil started in the southern states during 1970s with a movement against monoculture with the loss of genetic bio-diversity especially agro-biodiversity, along with soil erosion, water contamination and loss of capital in rural sector (Pereira, 2012).

10. Prevention of post-harvest losses

The fruit and vegetables (12-14%) are destroyed during the post-harvest activities due to improper handling, extreme temperature, high humidity, improper packaging and poor transportation and marketing (Asian Productivity Organization [APO], 2006). Recycled organic matter is simply used for packaging instead of plastic and paper bags in which large quantities of perishable fruits and vegetables can be stored. The main objectives of postharvest technology applications include quality maintenance (appearance, flavor and nutritive value), food safety and reduction of losses between harvest and consumption (Kitinoja & Kader, 1995). Simple, low cost, eco-friendly technologies can be more appropriate for limited resources stallholders to supply food items to the poor countries. Moreover, the growing demand for organically produced fruits and vegetables offer new skills for smallholder growers and traders (Kelly & Metelerkamp, 2015)

11. Organic farming and climate change mitigation

Organic agriculture has the capacity to mitigate greenhouse gases through nature friendly farming methods that enhance fertility of land and promote the use of natural substrates. The organic farming technologies consider organic management as relevant mitigation and adaption practices such as introduction of leguminous crop in to crop rotation, soil cover techniques, mixed and combined farming methods and sustainable agronomic practices (FAO, 2011c). A report published by environmental think-tank German watch stated that Pakistan was among the three states most affected by severe climate hazards during 2012 (Naeem, 2013).

CONCLUSION

Although, there is enough food available, everyone has the food, yet there are nearly 1 billion people worldwide who are facing hunger and another 1 billion are suffering from malnutrition owing to lack of
micronutrients they need to lead a healthy life. A majority of poor people cannot approach the food or access it. This is the situation of extreme poverty, natural disasters, conflict and war, poor infrastructure and overexploitation of the environmental resources. Organic farming is simple, cheap and effective in helping the poor communities around the world to have healthy food and better living conditions adapting the methods of good natural cures for the community. It is very important to know that all the farmers can improve their production with best appropriate agricultural techniques that are environment friendly. In addition, it is very important to advance emergency documentation and training under good biological technologies to ensure that smallholder farmers can have the best sustainable food production for the future human generations. Pakistan can learn from the Brazilian experience and flexible model combining the resources of all the provinces of Pakistan under the strong socio-political system.

**Author Contribution Statement** Abdul Sattar Anjum drafted the manuscript. Roshan Zada and Waqarul Hassan Tareen conceived the review and helped to draft the manuscript.

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Comparative study of tissue culture response of some selected basmati rice cultivars of Pakistan

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Key Message Four basmati rice cultivars of Pakistan were evaluated by their tissue culture responses. Basmati 370 and Super basmati were found to be efficient for callus formation and in vitro regeneration, respectively.

ABSTRACT This study was conducted to select the best tissue culture responsive basmati rice cultivar among Basmati 370, Basmati 385, Super basmati and Shaheen basmati. N6 and MS media having four levels of 2, 4-D (1.5, 2.0, 2.5 and 3.0 mg/l) and three levels of agar (4.0, 5.0 and 6.0 g/l) were used in order to evaluate the most appropriate level required for calli formation. Basmati 370 showed the best response as compared to other cultivars on both MS and N6 media. The most cultivars behaved well for callus induction at N6 media, 5.0 g/l agar and 2.0-2.5 mg/l 2, 4-D. Basmati 370 showed callus formation; 74.0-85.07%, Super basmati showed 65.5-75.17%, Basmati 380 showed 72-79% and Shaheen basmati showed 53.0-67.87% callus formation. For regeneration, various treatments (0.5/2.0, 1.0/4.0, 1.5/5.0 mg/l) of NAA/BAP were used. Super basmati exhibited the best response for regeneration (58.33%) at 1.0/5.0 NAA/BAP. Based on our findings, Super basmati was found to be the best tissue culture responsive cultivar. These results will be helpful in unveiling many aspects of callus induction and regeneration and have potential use in genetic improvement of rice by employing various techniques of genetic transformation including Agrobacterium-mediated transformation and gene gun method.

Keywords: Basmati 370, Basmati 385, Callus, Regeneration, Super basmati, Shaheen basmati

INTRODUCTION

Rice scientifically known as Oryza sativa (L.) is considered as a staple food crop for more than 50% population of the world, thus, making it a second most important cereal grain after wheat (Long-ping et al., 2014; Zahra et al., 2015; Akhter et al., 2015). It belongs to family Poaceae and has three major subspecies i.e. japonica, indica and javanica. The most cultivated subspecies in the world is indica that added 90% of total rice production of the world (Sharan et al., 2004). The three types of rice i.e. basmati, coarse, and short grain cold tolerant rice are being cultivated in Pakistan. After wheat, rice is 2nd staple food for the people of Pakistan that occupies approximately 2.58 million hectares with 5.54 million tons paddy production (Shakoor et al., 2015; Ahmed et al., 2015; Mahmood et al., 2016). Basmati rice has the best quality and aromatic which is exported to other countries (Rabani et al., 2010; Abbasi et al., 2015).

The production of rice is declining due to urbanization, environmental as well as biotic factors. It has been estimated that the population of the world will be increased up to 9 billion in 2050 (Kajala et al., 2011). These
circumstances warn us to improve the yield of rice through the latest biotechnological approaches in addition to conventional approaches so that the requirements will be fulfilled (Kajala et al., 2011).

Genetic transformation is one of the techniques of biotechnology which is being used by the researchers to improve the crop yield. This technique is dependent on tissue culture that is based on different factors such as source and type of explants, types of media, growth regulators, gelling agent and genotype (Joyia & Khan, 2013, Mehmood et al., 2013; Shah et al., 2013; 2014a, b; 2015a). The tissue culture of rice is genotype dependent.

Therefore, the current research study was planned to optimize the most suitable level of phytohormones and agar for calli induction and in vitro shoot regeneration and to select the most tissue culture responsive cultivar comparing four basmati cultivars (Basmati 370, Basmati 385, Super basmati and Shaheen basmati). Our findings will be useful for tissue culture of different other rice cultivars. The best tissue culture responsive cultivar can be used in genetic transformation experiments for the improvement of rice yield.

MATERIALS AND METHODS

Media preparation

MS basal media (Murashige & Skoog, 1962) and N6 media (Chu, 1978) enriched with four treatments of 2, 4-D (1.5, 2.0, 2.5 and 3.0 mg/l), and agar (4, 5 and 6 g/l) were prepared separately and used for callus induction. MS basal media was supplemented with different combinations of BAP/NAA (4.0/0.5, 5.0/1.0 and 6.0/1.5 mg/l) were used as regeneration media. pH 5.75-5.8 was maintained for each media. Subsequently, the media was sterilized by autoclaving at 121°C temperature for 20 min.

Sterilization of seeds

Mature seeds of four basmati cultivars; Basmati 370, Basmati 385, Super basmati and Shaheen basmati were de-husked, rinsed with autoclaved distilled water for dust removal. Ethanol (70%) was used to sterilize the seeds for 1 min time duration, and 50% Clorox (Sodium hypochlorite) was also used for fifteen minutes. Subsequently, the seeds were washed away 3-4 times with autoclaved water to eliminate the Clorox. At the end, the sterilized seeds were placed on tissue paper to suck the extra water.

Callus induction

The sterilized seeds were inoculated on callus induction media under controlled conditions for a period of two weeks. The propagated embryogenic calli were placed on maintenance media (callus induction media) for 3-4 days for further propagation.

Regeneration and acclimatization

After three weeks, the embryogenic calli were shifted on regeneration media for shoot formation. After shoot growth, plantlets were transferred to rooting media (MS media fortified with 0.5 mg/l NAA) for two weeks. The plantlets bearing roots transferred to tubes containing tap water for acclimatization for one week before shifting of plantlets to the soil. At the end, healthy plantlets were transferred to green house.

Statistical analysis

The study design was Completely Randomized Design (CRD). Data was recorded in percentage for each treatment and then ANOVA was employed. Means having significant differences were compared at P<0.05 with Duncan’s Multiple Range Test (DMRT) through M-STAT-C software.
RESULTS

Response of cultivars on callus induction media

The effect of cultivars on callus induction media has been shown in table 1. All the cultivars exhibited different responses showing significant differences with respect to callus induction frequency. Basmati 370 gave the best callus induction frequency on both MS and N6 media. Super basmati was significantly better than Shaheen basmati on both types of media. These results indicated that each cultivar had different capacity for callus induction; it may be due to difference in genetic makeup among different cultivars. In the present study, response of 2, 4-D for callus induction was checked in four cultivars of rice. The results revealed that 2.5 mg/l and 2.0 mg/l 2, 4-D were significantly better (P<0.05) for callus induction of rice cultivars than that of 1.5 mg/l and 3.0 mg/l 2, 4-D on MS media, while 2.5 mg/l 2, 4-D was found to be the best on N6 media (Table 2). It is clear from these results that the most basmati cultivars showed excellent response for callus formation on callus induction media supplemented with 2.0-2.5 mg/l 2, 4-D.

Response of cultivars on MS media fortified with different levels of agar

The table 3 indicated that Basmati 385 showed the best performance for callus formation (76.28%) at 5 g/l agar. Basmati 370 and Super basmati gave the maximum callus induction frequency (76.62 and 68.23%), respectively) at 6 g/l agar. Shaheen basmati revealed the best response of callus induction frequency (62.15%) at 4 g/l agar. However statistically there was no significant (P>0.05) effect among different levels of agar on callus induction in basmati cultivars (Table 3).

Response of cultivars on N6 media fortified with different levels of agar

The effect of agar on callus induction of basmati rice cultivars on N6 media has been shown in table 4. The results revealed that the highest callus induction frequency (85.07%) was obtained by Basmati 370 followed by Basmati 385 at 5 g/l agar, while Super basmati produced 75.17% callus induction frequency on N6 media fortified with 6 g/l agar. Hence, Basmati 370 was proved to be best cultivar for callus formation at 5 g/l agar. The results also indicated that less amount of agar (4 g/l) was not suitable for callus induction as compared to 5 and 6 g/l agar (Table 4).

Interaction of cultivars and MS-supplemented 2, 4-D media for calli formation

It was observed that there were no significant consequences for interaction of 2, 4-D and cultivars on callus formation on MS media because probability value was greater than that of 0.05 alpha level. Table 5 showed that Basmati 370 produced 82.99% callus induction at 2.0 mg/l 2, 4-D followed by Basmati 385 that yielded 76.97% callus induction at 2.5 mg/l 2, 4-D.

Interaction of cultivars and N6-supplemented 2, 4-D media for calli formation

Table 6 indicated that 68.74% calli was produced by Shaheen basmati, 82.4% by Basmati 385, 75.92% by Super basmati and 83.17% by Basmati 370 at 2.5 mg/l 2, 4-D. But statistically, interaction of 2, 4-D with cultivars had no significant (P>0.05) difference for callus induction on N6 media.

Regeneration of plantlets

Regeneration of all the cultivars under investigation was optimized on MS media having various combinations of NAA and BAP. The best regeneration response was achieved on MS media supplemented with 1.0 mg/l NAA and 5.0 mg/l BAP. The highest regeneration frequency (58.33%) was recorded in Super basmati followed by Shaheen basmati that gave 35% regeneration frequency on MS basal media having 1.0 mg/l NAA and 5.0 mg/l BAP. Green spots appeared on some calli and then shoots were regenerated from these green spots (Fig. 1). The results revealed that MS media supplemented with 1.0 mg/l NAA and 5.0 mg/l BAP was found to be optimum for successful regeneration and Super basmati showed the best regeneration response (Table 7). These plantlets were shifted on rooting media for roots formation.
Table 1: Response of basmati rice cultivars for callus induction (%) on MS and N6 basal media

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Callus induction frequency (%)</th>
<th>N6 basal media</th>
<th>MS basal media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basmati 370</td>
<td>77.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>75.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basmati 385</td>
<td>75.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>72.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Super basmati</td>
<td>70.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>66.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Shaheen basmati</td>
<td>61.74&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>60.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Similar superscript letters represent no significant differences, while diverse letters are exhibiting significant differences. DMRT was employed at 0.05 probability level. LSD value for MS media is 3.65, while that of N6 media is 3.21.

Table 2: Callus induction response (%) of basmati rice cultivars at different levels of 2, 4-D

<table>
<thead>
<tr>
<th>2, 4-D (mg/l)</th>
<th>Callus formation (%)</th>
<th>MS basal media</th>
<th>N6 basal media</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>63.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>64.60&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.0</td>
<td>72.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>73.80&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.5</td>
<td>72.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>77.57&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.0</td>
<td>65.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>69.67&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Similar superscript letters represent no significant differences. DMRT was employed at 0.05 probability level. LSD value for MS media is 3.654, while that of N6 media is 3.219.

Table 3: Callus induction response (%) of basmati rice cultivars on MS media enriched with different levels of agar

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Mean callus induction frequency (%)</th>
<th>4 g/l agar</th>
<th>5 g/l agar</th>
<th>6 g/l agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basmati 370</td>
<td>72.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Basmati 385</td>
<td>68.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Super basmati</td>
<td>63.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Shaheen basmati</td>
<td>62.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Similar superscript letters represent no significant differences. LSD value is 21.92 at 0.05 probability level.

Table 4: Callus induction response (%) of basmati rice cultivars on N6 media supplemented with different levels of agar

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Mean callus induction frequency (%)</th>
<th>4 g/l agar</th>
<th>5 g/l agar</th>
<th>6 g/l agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basmati 370</td>
<td>74.56&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>85.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.33&lt;sup&gt;bc&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Basmati 385</td>
<td>73.29&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>79.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.74&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Super basmati</td>
<td>65.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.39&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>75.17&lt;sup&gt;bc&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Shaheen basmati</td>
<td>53.12&lt;sup&gt;f&lt;/sup&gt;</td>
<td>67.87&lt;sup&gt;de&lt;/sup&gt;</td>
<td>64.24&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Similar superscript letters represent no significant differences. LSD value is 5.57 at 0.05 probability level.

Table 5: Interaction of 2, 4-D and basmati rice cultivars for callus induction (%) on MS media

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>2, 4-D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.5 mg/l</td>
</tr>
<tr>
<td>Basmati 370</td>
<td>68.82&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basmati 385</td>
<td>69.48&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Super basmati</td>
<td>62.03&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Shaheen basmati</td>
<td>54.63&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Similar superscript letters represent no significant differences. LSD value is 21.92 at 0.05 probability level.
Table 6 Interaction of 2, 4-D and basmati rice cultivars for callus induction (%) on N6 media

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>1.5 mg/l</th>
<th>2.0 mg/l</th>
<th>2.5 mg/l</th>
<th>3.0 mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basmati 370</td>
<td>69.25ab</td>
<td>81.28a</td>
<td>83.17a</td>
<td>78.24a</td>
</tr>
<tr>
<td>Basmati 385</td>
<td>71.11ab</td>
<td>73.41ab</td>
<td>82.45a</td>
<td>73.14ab</td>
</tr>
<tr>
<td>Super basmati</td>
<td>67.59ab</td>
<td>72.45ab</td>
<td>75.92a</td>
<td>67.59ab</td>
</tr>
<tr>
<td>Shaheen basmati</td>
<td>50.45b</td>
<td>68.06ab</td>
<td>68.74ab</td>
<td>59.72ab</td>
</tr>
</tbody>
</table>

LSD value is 19.31 at 0.05 probability level. Similar superscript letters represent no significant differences.

Table 7 Regeneration (%) on MS media enriched with NAA and BAP

<table>
<thead>
<tr>
<th>Varieties</th>
<th>NAA/BAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5/4.0 mg/l</td>
</tr>
<tr>
<td>Basmati 370</td>
<td>6.66g</td>
</tr>
<tr>
<td>Basmati 385</td>
<td>6.66g</td>
</tr>
<tr>
<td>Super basmati</td>
<td>18.33def</td>
</tr>
<tr>
<td>Shaheen basmati</td>
<td>16.66defg</td>
</tr>
</tbody>
</table>

Similar superscript letters represent no significant differences.
Fig. 1 (A) Callus induction (B) Calli showing green spots (C) Calli showing regeneration (D) Regeneration of plantlets
DISCUSSION

In present era of biotechnology, various techniques of genetic engineering are in practice throughout the world to improve genetic bases of cereals including rice. All these techniques primarily rely on successful regeneration of a healthy plant via tissue culture technique after transformation of an explant/callus phase (Bano et al., 2005; Shah et al., 2015b; 2016).

During this study, we found that all the cultivars under investigation showed higher percentage of callus induction on N6 medium as compared to MS media. Maximum percentage of calli were obtained at 2.0 mg/l and 2.5 mg/l 2, 4-D. Our findings were also confirmed by the previous research studies by Rashid et al. (1996); Rashid et al. (2001); Rashid et al. (2003); Sharan et al. (2004). Our findings are contradictory to Noor et al. (2005); Zaidi et al. (2006). These differences may be due to the use of different media, cultivars and their compositions. All the cultivars formed good quality calli at 5 g/l agar except Super basmati that gave good response at 6 g/l agar. Difference in callus induction frequency at the same concentration of 2, 4-D under similar conditions showed that callogenesis was affected by cultivar (Ali et al., 2004). Different research group investigated various factors affecting rice tissue culture including gelling effect such as agarose, phytagel and agar. In this research study, it was found that optimum levels of agar were suitable for embryogenic callus induction and proliferation, but its high concentrations favored the regeneration.

In regeneration experiment, regeneration media having different treatments of BAP/NAA were used. In this experiment, 1.0 mg/l NAA and 5.0 mg/l BAP was confirmed to be optimum for regeneration of calli. The most calli showed a positive response for shoot formation on regeneration media containing NAA and BAP. The role of auxins and cytokinins has been appreciated in plant tissue culture by Woodward et al. (2005). It was remarkable to note that the effect of cytokinins in tissue culture has been stressed more as compared to auxins. In previous studies, it has been reported that presence of any cytokinin like BAP or kinetin was inevitable for the regeneration medium (Kalaiarasi et al., 2014), while Pons et al. (2000) reported that in case of auxins like NAA and IAA, genotype played a dominant role for regeneration. In the present study, Super basmati showed the best response for shoot formation (58.33%) as compared to other cultivars. These results are supported by Rashid et al. (2000; 2001); Noor et al. (2005).

CONCLUSION

From this study, it is concluded that each cultivar has different response for callus induction and regeneration thereby each cultivar showed its own optimum level of 2, 4-D for the best callus formation. The level of 2, 4-D (2-2.5mg/l) was found to be the optimum level for the highest callus formation. This study also indicated that the very low level of 2, 4-D (1.5 mg/l) was not suitable for callus formation. Only optimum concentration favored the callus induction. Super basmati showed the best response for regeneration among all other cultivars. It would be considered as a model cultivar for tissue culture as well as transformation of genes. The finding of this study would be helpful for tissue culture of rice and its genetic improvement applying different transformation techniques. It is also suggested that Super basmati would be considered as a model cultivar for development of stress tolerance in rice through the latest biotechnology approach.

Author Contribution Statement Muhammad Arshad and Ghulam Muhammad Ali conceived and designed the research project. Khalid Mehmood conducted experiments and wrote the manuscript. Shaukat Ali contributed in optimizing various parameters for the evaluation of the best basmati rice cultivar i.e. technical assistance during the operation of experiments. Mazher Qayyum edited the article.

Conflict of Interest The authors declare that they have no conflict of interest.

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REFERENCES


Isolation and identification of *Agrobacterium tumefaciens* from the galls of peach tree

Nizar Ali¹, Akbar Zada¹, Murad Ali¹ and Zahid Hussain¹*

**Key Message** *Agrobacterium tumefaciens* attacks on peach plant and it causes crown gall disease. The present study was conducted to isolate and identify *Agrobacterium tumefaciens* from crown gall samples of peach plants.

**ABSTRACT** Peach (*Prunus persica*) is a very important fruit and it is attractive all over the world due to its delicious aroma and flavor. Peach is susceptible to various types of pathogens that result in decline of its fruit production. An important pathogen, *Agrobacterium tumefaciens* attacks on peach plant and it causes crown gall disease. The present study was conducted to identify the peach plants infected with *Agrobacterium tumefaciens* and isolate as well as identify the bacterium from the crown gall samples. These samples were collected from different locations including district Swat and Shangla. The bacterium was isolated from the samples using MacConkey selection media that is used specifically for identification of *Agrobacterium tumefaciens*. Two different biochemical tests i.e. Gram staining and KOH test were performed to confirm that the isolated bacterium is gram negative. The biochemical tests reveal that all the bacterial isolates are gram negative. Furthermore, two pathogenicity tests i.e. potato disc and carrot disc bioassay were conducted that confirmed the isolates causing tumors in the infected plant tissues. Antibiotic sensitivity tests reveal that the bacterial isolates are resistant to rifampicin antibiotic. All the morphological as well as the biochemical features of the bacterial isolates suggested that the samples isolated from crown galls were *Agrobacterium tumefaciens*. Our study provides the basis for further molecular characterization of the pathogens and to devise strategies for reducing the risk of bacterial infection and to enhance the yield of fruits.

**Keywords**: *Agrobacterium tumefaciens*, Antibiotics, Disc bioassay, Gram staining, KOH test

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**INTRODUCTION**

Peach (*Prunus persica*) belongs to Rosaceae family (Parveen et al., 2016). The production of peach ranked second among different fruits in Pakistan. Peach is a very important fruit because of its flavor, attractive colour, dietary value and medicinal worth. This fruit is enriched with carotenoids, ascorbic acid and phenolic compounds that act as antioxidants (Tomas-Barberan et al., 2001; Byrne, 2002). The peach was first grown in Asia, cultivated in Europe and later on it was introduced into Persia. In Pakistan, cultivation of peach occurs at about 15200 hectares and its annual production is 52600 tonnes (Agriculture Statistics of Pakistan, 2010-11). In Pakistan, the cultivation of peach occurs in different cities i.e. Peshawar, Swat valley, Quetta as well as many parts of Kohistan hills. More than 100 different families of dicotyledonous plants are infected by the gram negative bacteria called *Agrobacterium tumefaciens* (Lacroix & Citovsky, 2016). The bacterium own Ti-plasmid (Tumor inducing plasmid) that result in the formation of tumor at wound site in dicotyledonous peach tree (Roy, 2015). The size of Ti-plasmid is about 200 kb that carry 27 genes. The T-DNA region of the Ti-plasmid is excised, transformed into the host plant tissue and integrated into the genome of the plant (Kwon, 2016). The transformation of T-DNA and the subsequent formation of tumor require hormonal and virulence genes present on the Ti-plasmid (Kwon, 2016). The Ti-plasmid have region called transferred DNA
with tmr and tms genes and these genes are responsible for the over production of plant hormones such as cytokinins and auxins, respectively (Nester, 2015).

There are mainly two bacterial species namely *Agrobacterium vitis* and *Agrobacterium rubi* that are involved in the formation of crown gall disease. This disease produces tumor in plants and it is a very hazardous for the peach plants. The habitat of these pathogenic species is the soil (Roy, 2015). In dicotyledonous plants, the crown gall is caused by the *Agrobacterium* species that are polyphagous bacteria and are known as tumorigenic species of *Agrobacterium*. A huge loss occurs in walnut, pome, stone fruits and grapevine due to the crown gall disease. The cultivation of different host plants shows different levels of susceptibility. Some members like Arales and Liliales of monocots show susceptibility to this disease. Agrobacteria namely rhizogenes biovar 2 and *Agrobacteria* biovar 1 show a wider host range than that of *Agrobacterium vitis*, *Agrobacterium rubi* and *Agrobacterium larrymoorei*. Some strains show a wider host range, while others show to only one plant. The supervirulent strain shows a wide host range (Pulawska, 2010).

*Agrobacterium tumefaciens* has been isolated from different plant tissues including stem, leaf and crown gall samples of aster (Chen et al., 1999), from galls of apricot (Aysan et al., 2003), galls of rose (Aysan and Sahin, 2003), root nodules of *Vicia faba* (Tiwary et al., 2007) and tobacco (Furuya et al., 2004). Different selection media have been utilized for isolation of *A. tumefaciens* from plant samples including MacConkey media and yeast extract mannitol agar (YEMA) (Aysan et al., 2003; Aysan & Sahin, 2003). Crown galls have been isolated from different plant species and they belong to different dicotyledonous plants i.e. *Tectona grandis*, *Artocarpus heterophyllus*, *Anthcephalus codomba*, *Terminalis arjuna*, *Rosa chinensis* and *Solanum lycopersicum* (Sarker et al., 2011).

The characterization and identification of *Agrobacterium tumefaciens* is usually conducted by morphological, biochemical, pathogenicity, antibiotic sensitivity and molecular methods. The morphological methods include the observation based on size, shape, colony surface, colour, opaqueness, elevation, consistency and margin type. The biochemical method for bacterial identification is also method of choice for identification and confirmation of bacterial samples. The biochemical is usually conducted according to Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). In antibiotic sensitivity tests, the isolates are treated with antibiotics according to the Bauer Kirby method (Bauer et al., 1966) and confirm the bacterium by the formation of the inhibition zones. It shows resistance to some antibiotics, while it exhibits susceptibility to several other antibiotics. *A. tumefaciens* was also confirmed performing pathogenicity tests that were done on potatoes (*Solanum tuberosum*) (Hussain et al., 2007) and carrot (*Daucas carota*) (Aysan et al., 2003).

The crown gall disease limits the economic importance of dicotyledonous plants e.g. apple, rose, peer, almond and cherry (Pionnat et al., 1999). The tumor found in crown portion causes disruption of the vascular system as well as inferior development (Moore et al., 2001). The objectives of our study were isolation of *Agrobacterium* from crown galls samples of peach trees. We were also interested in cultural and biochemical characterization of the isolates.

The basic aim of this study was to isolate the virulent *Agrobacterium tumefaciens* from peach trees. We were also interested to confirm this bacterium by different morphological, biochemical, and pathogenicity and antibiotics sensitivity tests.

**MATERIALS AND METHODS**

**Crown galls collection**

Crown gall samples were isolated from the dicotyledonous plant i.e. peach (*Prunus perisca*). The samples were collected from the different areas of the district Swat and Shangla. These samples were taken into laboratory with great care to avoid contamination and used for sample isolation. The experimental period was during March to July, 2016.
Preparation of crown gall samples

The crown gall samples were labeled in the laboratory and rinsed with tap water to remove the hazardous materials and other soil particles from the samples. A solution of 10% commercially available bleach was prepared. The gall samples were immersed in the solution for 3-5 min but it depends on the galls nature. Subsequently, the galls were washed with sterilized distilled water to remove the traces of the bleach solution. The crown gall samples were kept in sterilized distilled water for 5-6 days to make it soft for sample collection. Then galls were chopped into small pieces and were kept in sterilized distilled water for 2-3 days.

MacConkey media preparation

The MacConkey media is composed of the following ingredients: 1.5 g bile salts, 17 g pancreatic digest of gelatin, 10 g lactose monohydrate, 3 g peptones (casein and meat), 13.5 g agar, 0.001 g crystal violet, 5 g NaCl, 0.03 g neutral red and pH 7.1 (Bopp et al., 1999). MacConkey media (49 g) was weighed according to the manufacturer instructions and dissolved in 1 liter distilled water. Then the solution was completely mixed and autoclaved for 15 min at 121 °C. The media was allowed to cool and then poured into petri plates under sterilized condition. The media in the petri plates were allowed to solidify and then sealed. The plates were kept for 24 h in the incubator at room temperature.

Crown gall extracts culturing on MacConkey media

Galls extracts were taken out from the sterilized water. The galls were cut into small pieces through a sterilized blade. Small pieces of samples were picked by a loop and inoculated into the MacConkey media by a Streak plate method. Two replicates were produced from each sample. Then petri plates were kept in the incubator at 28 °C for 2-3 days.

Sub culturing on MacConkey media

The initial bacterial culture was sub cultured on MacConkey media to produce pure culture of Agrobacterium. In the sub culturing, a single colony was picked from each plate with the help of loop and inoculated into the MacConkey media by a Streak plate method. The process was repeated continuously and from each plate, one replicate was formed. After sub culturing, the petri plates were kept in the incubator for two to three days at 28 °C. The pure culture appeared on MacConkey media that was used for different tests for the confirmation of Agrobacterium tumefaciens.

Identification of Agrobacterium tumefaciens

The bacterial species can be identified from certain biochemical and physiological characteristics by observing its physiological nature and morphological characteristics (Adenemo & Onilude, 2014). During this research study, the morphological characteristics such as texture, colour and shape of many bacterial colonies were taken into consideration. Different biochemical tests were performed to confirm the presence of Agrobacterium tumefaciens in galls. The biochemical tests such as potassium hydroxide test, gram staining, pathogenicity tests (carrot-disc bioassay and potato-disc bioassay) as well as antibiotic sensitivity test were carried out for the identification of Agrobacterium tumefaciens.

Potassium hydroxide test

In this test, 3% KOH solution was prepared. A single drop of KOH was put on a slide. Bacterial colony was picked from bacterial pure culture and mixed with KOH on the slides. The slides were rotated for 10-15 sec. Subsequently, the solution on the slide was picked up by tooth pick that revealed a sticky thread like paste. The formation of thread like paste confirmed that the isolated bacterial culture was gram negative.
Gram staining

The slide was washed with 95% ethanol. A drop of distilled water was placed on a slide. Then bacterial colony was picked from the bacterial culture, placed on the slide and properly mixed. The slides were air dried. Crystal violet dye was applied to the slide with the help of dropper for 30 sec. The slides were rinsed with sterile water to remove the excess dye. Gram Iodine was applied to the slide for 1 min and washed. Then 95% ethanol was applied and rinsed the slide again with the distilled water. The slides were further treated with Safranin called counter stain for 1 min and rinsed. After gram staining, our results showed red colour confirming the presence of gram negative bacteria.

Pathogenicity test

Carrot-disk bioassay and potato-disk bioassay were performed as pathogenicity test.

Carrot and potato-disc bioassays

In this test, carrot (Daucas carota) discs were used (Aysan et al., 2003). Carrot was cut into small disc and properly washed with 95% of Bleach solution for 3 min and then washed with double distilled water. Sterilized filter paper was kept on each petri-dish where the carrot disc was placed. A single colony of bacterial culture was picked up from petri plates and poured into each disc present in the petri dish. Subsequently, these plates were kept in incubator for 20 days to observe young galls formation on the carrot disc. The same method of carrot disc bioassay was repeated for potato disc (Hussain et al., 2007).

Antibiotic sensitivity tests of isolates

The antibiotic sensitivity tests were performed on the isolates according to the Bauer Kirby method (Bauer et al., 1966). The antibiotics namely cefotaxime, kanamycin, rifampicin and tetracycline were used. A standard bacterial culture (20 µl) was used in antibiotic sensitivity tests. A solution of 10 µl antibiotics was prepared and a filter paper was soaked in it and placed on the designated isolates. The plates were kept at 30 °C for 24 h in an incubator. The measurement of the size of inhibition zone shows vulnerability to antibiotics.

RESULTS

Collection of crown gall sample

The fresh crown gall samples were identified and collected from peach trees. The crown gall samples have been shown in fig.1.

Isolation of Agrobacterium tumefaciens

After inoculating crown gall samples on MacConkey media, the colonies of bacterial culture were noticed 2-3 days after inoculation at 28 °C. The initial bacterial culture plates have been shown in fig. 2. After sub-culturing of bacterial plates, the pure culture isolation was obtained. The inoculated plates showed the presence of Agrobacterium tumefaciens.

Identification of bacterium by morphological characteristics of the colonies

The morphological characteristics like shape, color and texture of the bacterial colonies were observed on MacConkey media. The shape of the bacterium was convex, colour of the bacteria was pink to brick red and the texture showed that colonies were smooth, circular, micoud, translucent and shiny appearance. All the observed characteristics were similar to that of Agrobacterium tumefaciens culture.
### Table 1 KOH tests of five isolates of bacterium

<table>
<thead>
<tr>
<th>Biochemical test</th>
<th>Isolate 1</th>
<th>Isolate 2</th>
<th>Isolate 3</th>
<th>Isolate 4</th>
<th>Isolate 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram staining</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
</tbody>
</table>

_ve_ = Gram negative

### Table 2 Gram staining of five isolates of bacterium

<table>
<thead>
<tr>
<th>Biochemical test</th>
<th>Isolate 1</th>
<th>Isolate 2</th>
<th>Isolate 3</th>
<th>Isolate 4</th>
<th>Isolate 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram staining</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
</tbody>
</table>

_ve_ = Gram negative

### Table 3 Carrot and Potato disc bioassay of five isolates of bacterium

<table>
<thead>
<tr>
<th>Pathogenicity test</th>
<th>Isolate 1</th>
<th>Isolate 2</th>
<th>Isolate 3</th>
<th>Isolate 4</th>
<th>Isolate 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrot-disc assay</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Potato-disc assay</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>

_+ve_ = Tumor formation in the discs
Fig. 1 Crown galls formation in different peach trees
Fig. 2 Agrobacterium tumefaciens isolated from crown galls on MacConkey media

Fig. 3 (A) KOH test of gram negative (B) Pathogenicity test of carrot disc assay
Biochemical tests for identification of bacterial isolates

Two different biochemical tests were conducted to confirm the identity of bacterium as gram negative.

Potassium hydroxide test

The KOH test was conducted in order to confirm that the bacterium was gram negative or positive. The thread like slime appearance of the smear of bacterial isolates after KOH test revealed that the isolated bacterial culture was gram negative (Table 1; Fig. 3A). The gram negative result further confirmed that the bacterial isolates were *Agrobacterium tumefaciens*.

Gram staining test

The isolated bacterial culture was further tested with gram staining to confirm the identity of the bacterium as gram negative. The color produced by the bacterial isolates after gram staining was red that further revealed similarity with *Agrobacterium tumefaciens* (Table 2).

Pathogenicity tests

Pathogenicity test with two different dicotyledonous plant samples i.e. carrot and potato were conducted to confirm the pathogenicity of *Agrobacterium tumefaciens* causing crown gall under *in vitro* conditions.

Carrot-disc and potato-disc bioassays

In both bioassay tests, the discs of both plants species were infected with the bacterial culture in lab conditions. The test was conducted to confirm whether the bacterium inoculated in the discs initiated the formation of galls. The results revealed that galls formation was initiated in all the petri dishes inoculated with bacterial isolates (Table 3). Hence, it was confirmed that the bacteria isolated were *Agrobacterium tumefaciens*. The discs with crown galls produced by bacterial isolates have been shown in fig. 3B.

Antibiotic sensitivity tests of isolates

The antibiotic sensitivity test was performed on the isolates whether the bacteria showed resistance or became vulnerable to these antibiotics. The bacterial isolates were found resistant to tetracycline and ampicillin and no zone of inhibition was formed around the respective antibiotic disc on petri dish by *Agrobacterium tumefaciens*. The two other antibiotics i.e. cefotaxime and kanamycin formed clear zone of inhibition around the discs and these zones showed that bacteria were vulnerable to these antibiotics.

DISCUSSION

In present study, we identified peach trees with crown gall disease most commonly caused by *Agrobacterium tumefaciens* and then we isolated the gall samples. The isolated bacteria from crown gall samples were confirmed as *A. tumefaciens* by different morphological, biochemical, and pathogenicity and antibiotics sensitivity tests.

Crown gall disease is caused by *A. tumefaciens* that is gram negative soil bacteria (Nester, 2015). *Agrobacterium tumefaciens* infects dicotyledonous plants; both herbaceous and woody plants (Rhouma et al., 2006). In dicots, *A. tumefaciens* is mostly present on the stem of the plants resulting in crown gall formation. Different methods have been used to isolate the bacterium from the galls including sample collection and culturing it on specific selection medium (Collins, 2001). In our study, we isolated the bacteria using MacConkey selection media. MacConkey media has been previously used as a selective media for the isolation of *Agrobacterium tumefaciens* from crown gall samples (Bopp et al., 1999). The culture colonies of *A. tumefaciens* showed brick red colour on MacConkey selection media (Bergey’s Manual of Determinative Bacteriology) (Holt et al., 1994). The bacterial isolates in our research study also revealed brick red color.
when cultured on MacConkey selection media that showed resemblance with the morphological features of *A. tumefaciens*.

We conducted different biochemical and pathogenicity tests including KOH test, gram staining, potato disc assay and carrot disc assay. All these tests revealed that the isolated bacterium was gram negative and had the ability to cause tumor in plant disc sample under *in vitro* conditions. The biochemical and pathological approaches have been authenticated in the previous research studies for the identification of *A. tumefaciens* from crown gall samples of different plant species (Chen et al., 1999).

We were interested to assess the sensitivity of *Agrobacterium tumefaciens*. Therefore, we conducted antibiotic sensitivity tests to appraise the sensitivity of this bacterium to various antibiotics i.e. tetracycline, rifampicin, cefotaxime and kanamycin using Bauer Kirby method. The bacterium exhibited natural resistance to tetracycline and rifampicin, while it was susceptible to cefotaxime and kanamycin. A similar type of study was conducted by Bauer et al. (1966) in which the bacterial samples showed resistance to tetracycline and rifampicin forming inhibition zones and were found susceptible to cefotaxime and kanamycin. The molecular identification of this bacterium by PCR is also the most efficient method of its identification (Koivunen et al., 2004; Rhouma et al., 2006). During this study we also confirmed the gram negative nature of *Agrobacterium tumefaciens* by different biochemical and pathogenicity tests that have also been reported by Chen et al. (1999).

**CONCLUSION AND RECOMMENDATIONS**

In the present study, we have identified crown gall formation in different fruit trees of peach. The bacterial pathogens causing crown gall disease was confirmed as *Agrobacterium tumefaciens* by different biochemical tests, pathogenicity tests and antibiotic sensitivity tests. The identification of the bacterium can help for future research to devise strategies for the control of this pathogen. Further study of this bacterium can also help to utilize it for genetic engineering purpose in plants. The following recommendations were made in the light of this project: The research should be broadening to other fruit trees like cherry, apricot etc. These bacterial isolates should be further confirmed by PCR.

**Author Contribution Statement** Nizar Ali and Akbar Zada carried out the experiment. Murad Khan helped during the experiment. Zahid Hussain participated in the design, arrangements and coordination of the study and helped to draft the manuscript. All the authors read and approved the final manuscript.

**Conflict of Interest** All the authors have approved the final version of the manuscript being submitted. The article is the authors’ original work. Our submitted manuscript is not under consideration for publication in another journal. The authors declare that they have no conflict of interest.

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**REFERENCES**


Phytochemical potentials and medicinal uses of twenty-four selected medicinal plants from Swabi, Pakistan

Muhammad Qasim1, 2*, Muhammad Khalid1, 5, Aqib Sayyed1, Ismail Din3, Kashif Hayat4, Rozina4 and Sohail Ahmad Jan4

Key Message This study describes the valuable ethno medicinal information about the ethno botanicals. During this study, twenty-four medicinal plant species were documented as herbal medicines by the communities of Swabi, Pakistan.

ABSTRACT Poverty is persistent in district Swabi, Pakistan. A majority of the population cultivates small farms for its survival and earns extra money adopting small trade of medicinal plants collection to prepare the herbal medicines. The current study is based on ethno botanical data collection of the study area to document the valuable ethno medicinal information for the future generation. It is a qualitative study of 24 selected medicinal plants by field visits and questionnaire. Plants were collected from three study sites (research stations, plain areas and hilly areas). The hill stations were surveyed every fifteen days from January, 2010 to December, 2010. Change in temperature of selected site is negligible but human activities, grazing and soil texture verify the plant diversity. Plant species grow well in hilly site than that of plain areas. The results showed that among twenty-four medicinal plant species documented as herbal medicines by Swabi communities, the most familiar species included *Hordeum vulgare*, *Lantana camara*, *Melia azedarach*, *Mentha longifolia*, *Morus nigra*, *Nerium odorum*, *Nicotiana tabacum*, *Opuntia dillenii*, *Oxalis corniculata* and *Phaseolus lunatus*. During this study, it was found that ethnomedicinal information is transferred from generation to generation from older to younger people. This study provides a base line for developing plans so that these precious species can be preserved and used for drug production in future. Over exploitation by unscientific way of harvesting of these species should be controlled. Moreover, tissue culture techniques should be used to increase the production of these plants.

Keywords: Ethno botanicals, Folks, Natural products, Swabi-Pakistan, Traditional medicines

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INTRODUCTION

Since the prehistoric times, plants have been used to protect human beings from serious diseases including cancer, neurodegenerative as well as microbial infections, because they possess various bioactive molecules which give multiple responses in various diseases (Napar et al., 2012; Ghali et al., 2014; Jan et al., 2015). They have not been only used as medicines but also provide the basic necessities of life such as shelter, clothing and food. The traditional system of medicines has been greatly improved by modern system of medicinal plants for the production of useful drugs. Ethno botanical study paved ways for the exploration of new classes of compounds (Gurib-Fakim, 2006). Medicinal plants provide sources of pharmaceuticals in order to treat diseases and hence their demand is increasing day by day. Trade initiated globalization of herbal medicines that unveiled the mystery of underlying metabolic mechanisms, novel ideas, diseases and settlements along with indigenous antipathy (Inoue & Craker, 2014). The common concern about production of medicinal
plants is progressively increasing every year at the domestic level. Due to more advanced techniques, the income levels have been increasing due to ample awareness among the growers about proper harvesting of medicinal plants. In the same way, research on natural products flourishes to make available plants in a sufficient quantity for the remedy of various diseases (Barnes et al., 2008; Oh et al., 2014).

From literature review, it has been found that the botanicals have been used conventionally for a long time to cure different forms of cancers but associated with fewer side effects as recorded in modern chemotherapy (Jung Park & Pezzuto, 2002; Cragg & Newman, 2005). Considering these potential benefits of plants as anticancer compounds, National Cancer Institute of USA conducted a detailed study on medicinal plants and collected 35,000 plant samples from twenty countries and evaluated 114,000 extracts for possessing anticancer activity (Shoeb, 2006). Prior to 1983, among ninety-two anticancer drugs marketed in USA were 60% of plant origins (Newman & Cragg, 2012).

The use of medicinal plants in Swabi is an alternative therapy which mainly depends upon indigenous knowledge obtained from ancestral experience. There is an important traditional botanical biography that describes the most frequently used plants in clinical conditions, but very few of them have been examined scientifically. However, the vast majority of botanicals have been still unexplored. Hitherto, their phytochemical and medicinal properties have not been validated. Keeping in view the above background, the present study was carried out to analyze taxonomic status, biochemistry and uses of twenty-four selected medicinal plants such as *Hordeum vulgare*, *Lantana camara*, *Melia azedarach*, *Mentha longifolia*, *Mentha viridis*, *Morus nigra*, *Nerium odoratum*, *Nicotiana tabacum*, *Opuntia dillenii*, *Oxalis corniculata*, *Phaseolus lunatus*, *Plantago ovata*, *Prunus amygdalus*, *Psidium guajava*, *Ricinus communis*, *Rosa indica*, *Silybum marianum*, *Solanum nigrum*, *Solanum xanthocarpum*, *Tamarix gallica*, *Trigonella foenum-graecum*, *Verbascum Thapsus*, *Vicia sativa*, *Vitis vinifera* from district Swabi, Khyber Pakhtunkhwa Pakistan. This is the first report about the medicinal uses of these botanicals based on the information gathered from elder people in Swabi, Pakistan.

**MATERIALS AND METHODS**

This research study was conducted in diverse areas of district Swabi, while the taxonomic and chemical constituent study was carried out at the laboratory of plant taxonomy, Abdul Wali Khan University, Mardan Khyber Pakhtunkhwa, Pakistan.

**Medicinal plants survey**

Before starting the investigation survey, an executive letter was obtained from Abdul Wali Khan University, Mardan, Pakistan, while verbal consent was received from each participant. After thorough study of literature, the study trips were planned keeping in mind the blooming period of medicinal plants. Two different methods were followed during fields. In first method, observations were kept visiting different localities. In second method, questionnaire was made to collect data in different villages from the herbal physicians, local inhabitants, timber dealers and drug dealers to gain the information about the different medicinal plants. People of age group above 40 years and local herbalists were considered for data documentation because they knew well the actual uses of medicinal plants in their folk knowledge. Questionnaire having questions related to the local names, local uses, economic importance and other relevant information were conducted to collect data. The questions about uses of plants, rate of utilization, availability and their market values were also discussed with the local people.

**Collection of medicinal plants**

The first trip was arranged to the plain areas of Swabi including Tarakai, Dagai, Ismaila, Zeda and Topai. Another trip was arranged to the hilly areas of Swabi including Sheraghund Hills, Naranje Hills and Shah Mansur Hills (Fig. 1). These study sites were chosen on the basis of information collected from traditional healers, community elders and health workers. During the medicinal plants collection, 3-5 specimens per plant were collected and their photographs were captured.
Medicinal plants preservation

After the medicinal plants collection, they were properly set on blotting papers and old newspaper for absorbing moisture in order to avoid plants spoilage, attack of fungi and rotting. The newspapers were changed after every 24 hours. The plants were dried and made them moisture free by this process for twenty days. The plants were also sprayed with 2% mercuric chloride (HgCl₂) as fungicides in order to prevent the fungal attack. The preservation process was completed in two months.

Specimen identification

Preliminary confirmation of medicinal plants was made in the field using manuals, while unidentified samples were recognized using taxonomic keys, experts and herbarium materials at laboratory of plant taxonomy, Abdul Wali Khan University, Mardan Khyber Pakhtunkhwa, Pakistan.

RESULTS AND DISCUSSION

The experimental and clinical information about botanicals suggest that district Swabi has diverse flora found in both hilly and plain areas (Fig. 2). In present study, four plants namely *Hordeum vulgare, Lantana camara, Melia azedarach* and *Rosa indica* had anticancer potentials along with other clinical uses. All the selected plants had multiple functions in the treatment of various diseases. The people of Swabi have been using these floras either as medicine or food, and hence these plants are known as central part of traditional pharmacopoeia. This study aimed to gather information on 24 Swabi medicinal plants with respect to ethnombotany, biochemistry and clinical uses (Table 1). The results showed the multiple uses of reported plants in the treatment of a lot number of diseases.

*Hordeum vulgare* is astringent, aphrodisiac, antilactagogue and stomachic. It is a natural remedy for catarrh and bronchitis. It is anti-cancer, anti-cholera and anti-cough. It is useful in the asthma and anemia ailments. It contains important compounds like β-glucan that is used against coronary heart diseases. The barley leaves have maximum amount of flavonoids and saponarin, and both of these compounds have strong antioxidant activities. It also contains an important essential element like magnesium, which serves as a co-factor for glucose metabolism and insulin level optimization (Lee et al., 2010; Kamiyama et al., 2012). *Lantana camara* oil is antiseptic for wounds and is useful for leprosy and scabies. Various human ailments such as cancer, ulcers, asthma and eczema are effectively controlled by the plant extracts of *Lantana camara*. It is used in high blood pressure. It is anti-malaria, anti-bilious fevers, anti-tetanus. It is also used in tumors, catarrhal infections and rheumatism. *Melia azedarach* leaves are used for leprosy and antilithic. Its root is resolvent, and seeds are used as antiurheumatism. It is used in sores and ulcers which don't have the tendency to heal. It is used for ringworm and scabies. The oil is insecticidal, antibacterial and used for malaria and leprosy. Alcoholic extract of stem bark is anticancerous, antispasmodic and antiviral. *Mentha longifolia* is antiseptic, beneficial in digestion. Its leaves and flowering stems have antiasthmatic and antidiarrhoeal properties. A tea from leaves is useful for fevers and headaches. *Mentha viridis* have expectorant, antiseptic, anti-bacterial potentials. It is used in headaches, rhinitis and sore throat. It is used in colic, cough and arthritis problems. It is also useful for alleviating swollen gums and mouth ulcers. It is anti-toothaches. The dry leaves are crushed, applied on the forehead in headaches. *Morus nigra* have analgesic, emollient, sedative, antibacterial properties and used in diabetes. The leaves are antibacterial, and used in colds and influenza. Eye infections and nosebleeds are treated with the dried leaves of *Morus nigra*. The stem is antirheumatic. Its fruit is laxative, nutritive and used in urinary incontinence, tinnitus and constipation in old age. Root bark is antitussive and used for asthmabronchitis and diabetes. *Nerium odorum* root and root-bark are powerful diuretic and cardiac tonic. *Nicotiana tabacum* is used for wound healing and headaches, curative for skin ailments, goiter, broken limes, headaches, ulcers, worms, syphilis and dropsy problems. Its various extracts are highly effective against gram positive and gram negative bacterial as well as many fungi species such as *Candida albican* and *Cryptococcus neoformans* (Maria et al., 2007). *Opuntia dillenii* is used in burning sensations, hepatitis and asthma. It is also used for ulcers, edema and leucorrhea. *Oxalis corniculata* is useful for dyspepsia, hemorrhoids, dysentery, diarrhea, dysmenorrhoea, amenorrhoea, hepatitis and burning sensation. The juice is used in stomach problems. The leaves are used as antiscorbutic. *Phaseolus lunatus* seeds provide protein and carbohydrates rich diet and used as astringent,
diuretic. Seeds contain cuisine which eliminates blood cholesterol, osteoporosis. *Plantago ovate* husk of the seeds is taken as serbat for dysentery, laxative, reduces blood cholesterol levels and much valued for digestive ailments. *Prunus amygdalus* seeds are stimulant, demulcent and narvine tonic; useful in impotency, constipation and skin disorders. The oils are slightly laxative; in combination with amla juice very beneficial in hair loss and dandruff. *Psidium guajava* leaves are anticough and used in toothache, ulcers, inflamed gums and chest ailments. Roots are more effective for cholera patients. *Ricinus communis* oil is used as mild purgative, lubricant, hair tonic and to remove pain of bone’s joints. If the oil is mixed with almond then it is useful for hair-restoration. Leaf decoction is useful in jaundice. *Rosa indica* is used for eye disorders, heart diseases, antifungal, anodyne, emmenagogue (flower) and anticancer (fruit). It is also used for stomach pains and swellings for the treatment of arthritis, boils and coughs.

![Map showing study sites of hilly and plain areas of Swabi, Pakistan](image)
Table 1. Taxonomic and phytochemical study of medicinal plants collected from hilly and plain areas of Swabi, Pakistan

<table>
<thead>
<tr>
<th>Botanical name</th>
<th>Local name</th>
<th>Phytochemicals</th>
<th>Medicinal uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hordeum vulgare</td>
<td>Jao</td>
<td>Protein, Fat, Carbohydrate, Fiber, Ash, Thiamine, Riboflavin, Niacin, Arginine, Histidine, Lysine, Tyrosine, Tryptophane, Phenylalanine, Cystine, Methionine, Threonine, Leucine</td>
<td>For Bronchitis, Burns, Cancer, Catarrh, Chest, Chilblains, Cholecystosis, Cholera, Cough, Debility, Diarrhea, Dyspepsia, Fever, Inflammation, Measles</td>
</tr>
<tr>
<td>Lantana camara</td>
<td>Panch phul</td>
<td>Pentacyclic Riterpenoids, Lantanoside, Lantanone, Lamicameric acid, Ursangilic acid, Oleocanic acid, Acetate, Tetracosanoic acid, Octadecanoic acid, Palmitic acid, Oleocanic acid, Oleananic acid</td>
<td>Plant extracts are used treatment of Chicken pox, Measles, Swellings, Eczema, Tumors, High blood pressure, Bilious fevers, Catarrhal infections, Tetanus, Rheumatism, Malaria</td>
</tr>
<tr>
<td>Melia azedarach</td>
<td>Bakain</td>
<td>Bakayanin, Rutin, Quercitrin, Backalactone, Cystine, Serine, Arginine, Glycin, Glutamic acid, Proline</td>
<td>Leaves, root, seed: For Leprosy, Scrofula, Antilithic, Diuretic, Deobstruent, Resolvent, Sores, Ulcers, Ringworm, Scabies, Malaria fever</td>
</tr>
<tr>
<td>Mentha longifolia</td>
<td>Velane</td>
<td>Volatile oil, Thymole, Resine, Tannin, Gum, Pulegone, Isomethone, Borneol, Piperitenone oxide</td>
<td>Leaves, flowering stem: Antiasthmatic, Antispasmodic, Antidiarrhoeal, Carminative, Fevers, Headaches, Digestive disorders</td>
</tr>
<tr>
<td>Morus nigra</td>
<td>Tooth siah</td>
<td>Hallucinogens, Hydroxyresveratrol, Dihydromor, Morin, Sanggenol, β-sitosterol</td>
<td>Leaves, stem, fruit, root bark: For Diabetes. Colds, Influenza, Eye infections</td>
</tr>
<tr>
<td>Nerium odorum</td>
<td>Gandere</td>
<td>Glucoside, Rosagine, Tannic acid, Wax</td>
<td>Root, Root-bark: powerful diuretic and cardiac tonic</td>
</tr>
<tr>
<td>Nicotiana tabaccum</td>
<td>Tamakoo</td>
<td>Nicotine, Nicotinan, Nicotine, Nicoteine, Nicoteline, Cholin, Anabasine, Ntabasine</td>
<td>Leaves: For wound healing and headaches, curative for skin ailments, sedative, diuretic, expectorant, discutient, salagogue, internally only as an emetic, goiter</td>
</tr>
<tr>
<td>Opuntia dillenii</td>
<td>Zukam</td>
<td>Opuntiol, Ferulic acid, Hydroxybenzoic acid, Malic acid, 3-O-methyl isorhamnein, 1-Heptanecanol, Vanillic acid, Isorhamnetin-3-O-beta-D-rutinoside</td>
<td>For burning sensations, Whooping cough, Hepatitis, Asthma, Poison, Fever, Constipation, Conjunctivitis, Edema, Leucorrhoea, Menorrhagia</td>
</tr>
<tr>
<td>Oxalis corniculata</td>
<td>Khatibuti</td>
<td>Oxalic acid, Tannins, Potassium, Palmitic acid, Vitamin C</td>
<td>Leaves, Juice of plant: Used for Dyspepsia, Hemorrhoids, Dysentery, Diarrhea</td>
</tr>
<tr>
<td>Phaseolus lunatus</td>
<td>Lobyia</td>
<td>Phaseolunatin Cuisine, Carbohydrate, Protein, Lectin, Phytin, Tannin, Linamarin, Glucoside, Folic acid, Manganese, Vitamin B</td>
<td>Leaves, stems, seeds: Used as astringent, diuretic, seeds contain cuisine which eliminate blood cholesterol, while manganese prevent osteoporosis</td>
</tr>
<tr>
<td>Plantago ovate</td>
<td>Espaghoul</td>
<td>Protein, Mucilage, Fiber, Albumin, Globulin, Prolamin, Linoleic acid, Oleic acid, Xylose, Arabinose, Galacturonic acid, Rhanose, Galactose, Albumin, Tannin, Acetylecroline</td>
<td>Plantago ovate husk of the seeds taken as serbat for dysentery, Laxative, reduces blood cholesterol levels and much valued for digestive ailments</td>
</tr>
<tr>
<td>Prunus amygdalus</td>
<td>Badam</td>
<td>Fixed oil, Mucilage, Sugar, Iron, Phosphorus, Calcium, Niacin, Sphingolipid, β-d-glucopyranosyl, β-sitosterol, Daucosterol, Uridine,</td>
<td>Seeds, Oil: Stimulant, Demulcent, Narvive tonic, useful in impotency, constipation and also use for skin disorders. The</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Part Used</td>
<td>Chemical Constituents</td>
<td>Uses</td>
</tr>
<tr>
<td>----------------------------</td>
<td>-------------------------</td>
<td>---------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Psidium guajava</strong></td>
<td>Amrood</td>
<td>Ascorbic acid, Iron, Pentacyclic, Triterpenoid, Guajanoic acid, β-sitosterol, Uvaol, Oleanolic acid, Ursolic acid</td>
<td>Roots, Bark, Leaves, Immature fruits: To halt gastroenteritis, Diarrhea, Dysentery</td>
</tr>
<tr>
<td><strong>Ricinus communis</strong></td>
<td>Arand</td>
<td>Alkaloid ricinine, Toxalbumin, Lipids, Fixed oil, Triricinolin</td>
<td>Oil used as mild purgative, lubricant and hair tonic</td>
</tr>
<tr>
<td><strong>Rosa indica</strong></td>
<td>Surkh gulab</td>
<td>Fresh flowers of Rosa indica yields 0.013 to 0.15% essential oil. Composition of this essential oil is 22.1% stearoartenas, 16.36% phenethyl alcohol, 12.78% geraniol, 23.39% citronellol and gallic acid</td>
<td>Fresh flowers: Used for eye disorders, heart diseases, antifungal, anodyne, emmenagogue, anticancer, stomach pains and swellings for the treatment of arthritis, boils and coughs</td>
</tr>
<tr>
<td><strong>Silybum marianum</strong></td>
<td>Kariza</td>
<td>Flavone Lignans, Linoleic acid, Oleic acid, Palmitic acid, Protein, Stigmasterol, Sitosterol;</td>
<td>It is used in cirrhosis, jaundice and hepatitis ailments.</td>
</tr>
<tr>
<td><strong>Solanum nigrum</strong></td>
<td>Kachmac hoo</td>
<td>Alkaloid, Solanine, Solanidine, Saponin, Alpha, Beta Gamma Chaconines, Alpha, Beta Gamma Solanines</td>
<td>It is used in spleen enlargement, and root acts as a useful drug in hepatitis C. It is a good anti rheumatism drug.</td>
</tr>
<tr>
<td><strong>Solanum xanthocarpum</strong></td>
<td>Maraghu ne</td>
<td>Steroidal alkaloid; Solasodine, Glycoalkaid, Diosgenin, Alcoholic extracts. Fruit contains Solasonine, Sterols, Carpesterol</td>
<td>Its root is used for catarrh, asthma and bronchospasm, while fruit and root are good drug for flatulence and toothache</td>
</tr>
<tr>
<td><strong>Tamarix gallica</strong></td>
<td>Ghazz</td>
<td>Alkaloid, Tamarixin, Aglocone, Tamarixetin.</td>
<td>Tamarix gallica is used in healing of wounds.</td>
</tr>
<tr>
<td><strong>Trigonella foenum-graecum</strong></td>
<td>Malhuz</td>
<td>Mucilage, Calcium, Phosphorus, Iron. Protoalkaloids, Trigonelline, Choline, Saponins</td>
<td>It is used in digestive and menstrual pains. It increases breast milk. It is used to reduce cholesterol level in blood and sugar in urine.</td>
</tr>
<tr>
<td><strong>Verbascum Thapsus</strong></td>
<td>Volatile oil, Saponin, Amaroid, Gum, Mucilage, Resins</td>
<td>It is an expectorant, as well as an anodyne. Its leaves and flowers are used in hay fever. It is used in respiratory, coughs and colds problems.</td>
<td></td>
</tr>
<tr>
<td><strong>Vicia sativa</strong></td>
<td>Chelu</td>
<td>Volatile oil, Protein, Ash, Fiber, Aliphatic hydrocarbons, Aldehydes, Ketones</td>
<td>Vicia sativa seed is useful for emollient.</td>
</tr>
<tr>
<td><strong>Vitis vinifera</strong></td>
<td>Angoor</td>
<td>Oxalic acid, Malic acid, Tartaric acid, Linoleic acid, Oleic acid, Palmitic acid, Stearic acid, Palmitoleic acid, Phenols, Tocopherols, Steroids, Campesterol, Beta-sitosterol, Stigmasterol, Vitamin E</td>
<td>It is used as astringent, while fruit has cooling, stomachic and laxative properties. Fruit is also used in cardiac pain and heart palpitation and acts as an excellent source of blood builder</td>
</tr>
</tbody>
</table>
Fig. 2 Identification and collection of 24 selected medicinal plants from hilly and plain areas of Swabi (A) Hordeum vulgare (B) Lantana camara (C) Melia azedarach (D) Mentha longifolia (E) Mentha viridis (F) Morus nigra (G) Nerium odorum (H) Nicotiana tabacum (I) Opuntia dillenii (J) Oxalis corniculata (K) Phaseolus lunatus (L) Plantago ovata (M) Prunus amygdalus (N) Psidium guajava (O) Ricinus communis (P) Rosa indica (Q) Silybum marianum (R) Solanum nigrum (S) Solanum xanthocarpum (T) Tamarix gallica (U) Trigonella foenum-graecum (V) Verbascum thapsus (W) Vicia sativa (X) Vitis vinifera
Silybum marianum is used in cirrhosis, jaundice and hepatitis ailments. Solanum nigrum is used in spleen enlargement, and root acts as a useful drug in hepatitis C. It is a good anti rheumatism drug. Solanum xanthocarpum’s root is used for catarrh, asthma and bronchospasm, while fruit and root are good drug for flatulence and toothache. Fruit are also useful for antihelmintic and indigestion. Tamarix gallica is antifoul, antulcers and antidiarrhoea. It is also used in healing of wounds. Trigonella foenum-graecum is used in digestive and menstrual pains. It is used to reduce cholesterol level in blood and sugar in urine. Seeds are used in aphrodisiacs.

Verbascum thapsus is an expectorant as well as an anodyne. Its leaves and flowers are used in hay fever. It is used in respiratory, coughs and colds problems. It is antiemphysema and effective in asthma. The roots are boiled and used in bladder incontinence. Oil is used for ear infections, hemorrhoids and bronchial inflammation. Oil is also used as an effective drug for chest congestion, swollen joints and arthritis. Vicia sativa seed is useful for emollient. Vitis vinifera is used as astringent, while fruit has cooling, stomachic and laxative properties. Fruit is also used in cardiac pain and heart palpitation and acts as an excellent source of blood builder.

A similar type of research study was carried out by investigating the effects of Chinese medicinal plants in China for the treatment of various human ailments. It has been documented in this study that previous research notes and ethnobotanical surveys reported plant remedies for the treatment of diabetes and concluded that these ancient Chinese medical articles and ethnobotanical surveys provided a base line for drug discovery to alleviate epidemic diabetes at global level (Ma et al., 2014). Similarly, Hussain et al. (2013) conducted a detailed study about medicinal plants of Parachinar (FATA) Khyber Pakhtunkhwa, Pakistan based on folk knowledge to motivate the local people of Parachinar to use botanicals in an efficient manner. In their study, it was reported that area had high diversity as well as precious medicinal plants that have been used traditionally to treat various human ailments. Kidane et al. (2014) investigated the use of botanicals in Southern Ethiopia for the treatment of human health issues and reported that about 80% of inhabitants of Ethiopia relied on medicinal plants for curing various diseases. In their study, qualitative and quantitative field survey, and individual and group discussions were followed to generate scientific data about testing to verify the efficacy and dosage of medicinal plants to alleviate major threats to human health. Abera (2014) scrutinized the effects of medicinal plants of district Ghimbi, Southwest Ethiopia and documented that ethnobotany is a real and rich biological source for the identification, certification, status and proper usage of medicinal plants. This research group collected information about medicinal plants by field observation and interviewing 30 key informants and 165 local members and ascertained that 49 botanicals were used to treat various human diseases in Ethiopia.

The same nature of research study was conducted in district Swat, Pakistan in which local farmers, collectors and dealers were interviewed about the collection efforts, number of botanicals collected, price obtained and their net income from medicinal plants. From this research survey, it was noticed that collection of wild plants was only source of botanicals raw material for human ailments, with actually no cultivation. It was inferred that medicinal plants had great economic benefits but there were some drawbacks in market which could be addressed by proper knowledge of medicinal plants, efficient linkages in market chain and appropriate harvesting practices (Sher et al., 2014).

CONCLUSION

This study aimed to collect the wild sources of medicinal plants in Swabi, Pakistan. The results of this study corroborated the large population of medicinal plants that were more effective against various human ailments. The present data could be a significant part of both pharmaceutical industries of Pakistan and local farms that specialized in medicinal plants cultivation. These botanicals possessing medicinal properties might provide a basic raw material for making huge medicinal preparations. This raw material of botanicals should be produced at standard quality that would allow us to estimate and control these plants during cultivation and selection.
Author Contribution Statement  Muhammad Qasim conducted the research study. Muhammad Khalid and Aqib Sayyed helped in survey and collecting information. Ismail din contributed in plants collection and preservation. Kashif Hayat analyzed the data. Sohail Ahmad Jan wrote the manuscript. Rozina edited the manuscript. All the authors have read and approved the manuscript.

Conflict of Interest  The authors declare that they have no conflict of interest.

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