Pak. J. Agri. Sci., Vol. 50(1), 83-89; 2013
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
http://www.pakjas.com.pk

MANGO LEAF GALL FORMATION: VARIETAL SUSCEPTIBILITY AND WITHIN TREE DISTRIBUTION

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The present study was carried out to screen most commonly cultivated mango, *Mangifera indica* L., cultivars for their susceptibility to gall formation. Sarooli cultivar proved to be the most resistant one by having a minimum number of galls per 100 leaves. The abundance of galls in four quadrants of the tree i.e., east, west, north and south, was also studied which revealed that east quadrant had maximum number of galls while the abundance of galls in the remaining quadrants was variable. Gall formation on mango leaves seemed to increase gradually with increasing height from the ground level, reached a maximum at the height 12 ft to 16 ft and then declined. Leaf area measurements and nutrient analysis of the leaves were also done to see their impact on gall formation. Correlation analysis revealed that gall formation was positively linked with leaf area and the amount of Zn (ppm), P (%), K (%) while N (%) had negative correlation ($P<0.05$) with gall formation. In conclusion, the findings of the present study could be helpful in the management of mango leaf gall formation.

Keywords: *Mangifera indica*, leaf gall formation, gall formers, mango.

INTRODUCTION

The nutritional status of the host plant has significant influence on the ecology, behavior and physiology of herbivores (Schoonhoven *et al.*, 1998). There is an increasing trend to explore the plant-herbivore interactions in the last few decades, particularly the insect-host plant interactions (Lebel *et al.*, 2008). Resultantly, a number of hypotheses have been proposed worldwide to explain such relations (Dodge *et al.*, 1990; Ferguson *et al.*, 1991). The arthropods, for example galling insects, are mobile for a short period and usually do not change location after oviposition choice, it becomes visible that colonization of specific leaves or shoots within the host is imperative, since all plant parts can not respond to the insect attack (Burstein and Wool, 1993). Galling insects are specialist plant feeders with most species confined to a particular host. Many studies propose that the selection of plant parts by gall forming agents have an important role for the survival of their immature within a host plant population, or within a particular plant in that population (Craig *et al.*, 1989; Fernandes and Price 1991). In this process, females of gall forming communities select suitable plants and their parts which usually depend on the physiological or nutritional status of the plants. This selection behavior is essential so as to maximize nutrition of their young ones (Weis *et al.*, 1988; Rohfritsh, 1992) or to protect them from microclimatic stresses (Edward and Wratten, 1980). Many researchers have suggested that the nutritional status of the host plants could affect the insect-herbivore interactions (Mattson, 1980; Scriber, 1984; Slansky and Rodriguez, 1987; Khan, 2010). Besides physiological factors, morphological factors like host plant architecture i.e., plant canopy, height, vigor, number and area of leaves and crown volume (Holb *et al.*, 2001), are also critical in determining herbivore or insect-plant interactions (Espirito-Santo *et al.*, 2007). In addition, spatial distribution of host could also influence these interactions (Marques *et al.*, 2000).

Mango (*Mangifera indica* L.) is an important fruit crop of Asia and is widely found in different parts of Pakistan (Khan *et al.*, 2010). There are a number of insects associated with mango which includes mango mealy bug, fruit flies, mango hoppers and gall inducing insects, causing immature falling of fruits by depriving the plants' vital nutrients (Karar *et al.*, 2006). Mango gall formation is becoming an emerging threat to mangoes in South Asia particularly in Pakistan (Anonymous, 2010). Sharma (2009) provided a checklist of gall forming insects from India but to date there is a lack of basic knowledge, i.e. eco-biology, taxonomy, behavior and management, about the gall forming insects in Pakistan (Soomro *et al.*, 1987; Khan *et al.*, 2010). The present study was done with the objectives to find the varietal preference and within tree distributional patterns of gall forming insects on mango and the factors mediating their abundance.

MATERIAL AND METHODS

The present study was conducted in a farmer's field in mango orchard Multan (30°12′0″N, 71°25′0″E) where climatic conditions are arid sub-tropical continental with a hot summer and meek winter. The temperature fluctuates around 8-12^{o}C and 38- 50^{o}C as minimum and maximum, respectively.

***Experimental material and layout*:** Six mango cultivars viz., Anwar Ratool, Chaunsa, Dusehri, Fajri, Sindhri and Sarooli, were selected for experiments. The experiments were designed following randomized complete block design (RCBD) with five replicates. The experimental field was divided into five blocks and from each block one tree of each cultivar, relatively of uniform age (5-8 years), was selected randomly under more or less uniform conditions of soil fertility, irrigation and other cultural operations. Different mango cultivars were considered as factors and each tree under study as a replication.

The insects that fabricate the galls are rarely observed because most of the gall forming insects are extremely small. Yet, the actual galls can readily be observed. The attack usually occurs in the spring season (March-May) before the leaves are fully developed (Karar *et al.,* 2006; Anonymous, 2009a). Therefore, the data were collected at the end of spring season.

***Varietal preference*:** As the light effects were not uniform in different directions of the tree, each tree of the subjected mango cultivars was visually divided into four directions or quadrants viz., east, west, north and south (Aghajanzadeh and Mallik, 2007). The sampling unit was the leaf with a sampling size of 100 leaves per replicate. Twenty five leaves were collected randomly from each quadrant and no. of galls was counted from the collected leaves.

***Within tree distribution*:** Each quadrant of the subjected tree was further divided visually into five different levels from the ground viz., 4, 8, 12, 16 and 20 ft. Five leaves were collected randomly from each height by using bar stool, and no. of galls from the collected leaves were counted.

***Morphological and physiological factors*:** Mean leaf area was studied as a morphological factor for the purpose to see the relationship between leaf area and the insect attack. Twenty leaves/cultivar (5 leaves per quadrant) in five replicates were taken randomly. Leaf area was measured with the help of a leaf area meter.

Among physiological factors, the nutrient status of the cultivars was assessed after leaf analysis as only the plant's nutritional analysis can discern the actual nutrient status of a plant or crop (Anonymous, 2009b). Twenty four leaves/cultivar were taken randomly. The leaves were selected from the end of the branch, the third and fourth leaf from the terminal bud (Poffley and Owens, 2005) and analysis was done in Soil and Water testing Laboratory, Multan.

Nitrogen was quantified by Gunning and Hibbard's method of sulfuric acid digestion by taking one gram of plant material. The distillation was done with micro-Kjeldahl's apparatus (Jackson, 1962).

To quantify Phosphorus and potassium, known weight of leaf samples was digested in a di-acid mixture (HNO_3, $HC1O_4$) and reading the K element on Jenway PFP 1 Flame Photometer (Method 10a and 11a). Phosphorus was analyzed with developing vanadomolybdo-phosphoric acid yellow color. The intensity of the color was measured on spectronic 20 (Bausch and Lomb) at 470 nm wave length.

Zinc, iron, copper and manganese were quantified by digesting the known weight of samples in a di-acid mixture of (HNO_3, $HC1O_4$) and recording the elements on AAS 6 Vario Atomic Absorption Spectrophotometer (Baker and Amacher, 1982).

***Statistical analysis*:** Kolmogorov Smirnov test (normality test) and Levene's test (variance test) was applied before any statistical analysis. To meet normality and homoscedasticity assumptions of the analysis of variance (ANOVA), most of the data was transformed to square root transformation $(X+0.5)^{1/2}$ (Gomes and Gomes, 1984). When all the assumptions of ANOVA were satisfied, the data were analyzed by following ANOVA (Analytical Software, 2005). Multivariate regression technique and Pearson's product movement correlation were applied to check the dependence of gall formers on different leaf parameters.

RESULTS

Varietal preference and within tree distribution of galls: Mango leaf galls were of variable shapes and types i.e. globular, blister, circular etc. but these types are not discussed here. The leaf galls are formed by a still not described species of Cecidomyiidae (Diptera) in Pakistan. Pooled analysis of variance of the data regarding total no. of galls in all the quadrants of mango cultivars revealed a highly significant interaction between quadrants and cultivars ($F=39.16$, $df=15$, $P<0.01$). The main effects of the cultivars and quadrants were also significant ($F=2472.05$, $df=5$, $P<0.01$; $F=183.59$, $df=3$, $P<0.01$ respectively) (Table 1). LSD test on two-way ANOVA showed that Fajri was the most susceptible cultivar while Sarooli proved to be the most resistant cultivar, in all the four quadrants. East quadrant, in all the cultivars, received maximum no. of galls per 25 leaves while in Sarooli all the quadrants were statistically at par with respect to no. of galls per 25 leaves (Table 2).

Heights from the ground level play a significant role in gall abundance. All the mango cultivars, except Sarooli, showed a highly significant interaction between tree quadrants and height from the ground level ($F=19.10$, $df=12$, $P<0.01$ for Anwar retool; $F=11.06$, $df=12$, $P<0.01$ for Chaunsa; $F=5.22$, $df=12$, $P<0.01$ for Dosehri; $F=3.18$, $df=12$, $P<0.05$ for Fajri; $F=5.79$, $df=12$, $P<0.01$ for Sindri; $F=1.26$, $df=12$, $P>0.05$ for Sarooli). The no. of galls per 5 leaves in almost all the

Table 1. Mean comparison of the data showing significant interaction between the cultivars and different quadrants

Mango cultivar	Mean no. of galls/100 leaves[a]			
	East	West	North	South
Anwar ratool	126.4d A	58.2c B	55.6c B	28.6d B
Chaunsa	211c A	155.4b B	152.2b B	128.2c B
Dosehri	213c A	75.8c B	181b A	101.8c B
Fajri	1045a A	833.4a B	892a B	604.4a C
Sindri	377b A	77.6c D	157b C	213b B
Sarooli	32e A	22e A	22c A	10.4d A

[a]The values are averages of five replications. Values within each column or row followed by different letters are significantly different by using two-way ANOVA and LSD. Lower case letters immediately following values represent comparisons within a column and capital letters represent comparisons within a row. $LSD_{0.05}$ value for comparing means is 34.23 galls/100 leaves.

Table 2. A multivariate regression model for different nutrients from the leaves affecting gall formation

Regression Equations	$R^2_{Adj.}$ (%)	Role of individual factor (%)
Y = 16485.6-8623.98X1	66.96**	66.96 X1
Y = 1299.80-8623.98X1+13038.6X2	97.15**	30.19 X2
Y = 1542.02-606.927X1+14899.0X2-867.55X3	97.25*	0.10 X3
Y = 4955.91-1208.38X1+18324.3X2-1922.32X3-115.59X4	97.36*	0.11 X4
Y = 4113.19-3171.93X1+19670.2X2-3410.57X3-88.3971X4+34.22X5	98.50**	0.14 X5
Y = 4077.57-3553.29X1+18355.9X2-3103.64X3-84.1528X4+34.93X5+18.0421X6	98.95**	0.45 X6
Y = 3354.26-3222.60X1+17755.1X2-3629.52X3-115.26X4-02.463X5+31.5804X6+871.330X7	98.96**	0.01 X7

X1=nitrogen (%); X2=phosphorus (%);X6=manganese (ppm); X3=potassium (%);X4=zinc (ppm); X5=iron (ppm); X6=manganese (ppm); X7=copper (ppm); * Significant at P<0.05; ** Significant at P<0.01

quadrants, except Sarooli, gradually increased with increasing height from the ground level, reached maximum at heights 12ft and 16ft and then declined (LSD, P<0.05) (Fig. 1).

***Nutrient analyses*:** Nutritional status of the leaves varied significantly among all the mango cultivars. The maximum percentage of nitrogen was found in the leaves of Dosehri and Sarooli cultivars while the minimum percentage was found in Fajri (F=70. 4, df=5, P<0.01) (Fig. 2a). Maximum percentage of phosphorus was found in Fajri while the minimum was found in Dosehri and Anwar retool, the latter both were statistically at par (F=41.0, df=5, P<0.01) (Fig. 2a). Potassium (%) was quantified maximum in Fajri while the minimum was found in Anwar retool followed by Chaunsa and Dosehri, both were statistically at par (F=33.30, df=5, P<0.01) (Fig. 2a). Maximum iron (ppm) was present in leaves of Sarooli followed by Sindri and Dosehri, both the latter were statistically at par, while least was found in leaves of Chaunsa followed by Fajri cultivar (F=16989, df=5, P<0.01) (Fig. 2b). Maximum copper (ppm) was found in leaves of Fajri cultivar while least was found in leaves of Chaunsa cultivar (F=21.40, df=5, P<0.01) (Fig. 2b). Dosehri leaves were found with the maximum amount (ppm) of copper followed by Fajri while Sindri had minimum amount of copper (F=740, df=5, P<0.01) (Fig. 2b). Leaves of Fajri had maximum amount of zinc (ppm) followed by leaves of Anwar ratool and Chaunsa cultivars while leaves of Dosehri, Sindri and Sarooli had minimum amount of Zinc (ppm) and were statistically at par (F=31.60, df=5, P<0.01) (Fig. 2b).

***Leaf area* (m^2):** All the tested mango cultivars were highly significantly different from each other with respect to leaf area measurements (F=527, df=5, P<0.01). Fajri cultivar leaves had larger leaf area (m^2) followed by Sindri, while Sarooli and Anwar ratool had smaller leaf area (m^2) (Fig. 4)

Simple correlation was worked out between leaf parameters and abundance of galls in different mango cultivars (Fig. 4). Leaf area, zinc, phosphorus and potassium were found to be positively correlated (P<0.05) while nitrogen had a significant negative correlation (P<0.05) with the abundance of galls. Manganese, copper and iron had non significant correlation with the abundance of galls (P>0.05).

A multivariate regression model was developed for the purpose to determine the role of individual plant nutrient in gall abundance in different mango cultivars (Table 2). Nitrogen showed a major role (66.96%) followed by phosphorus (30.19%). The co-efficient of determination (R^2) increased by adding each nutrient in the model and reached

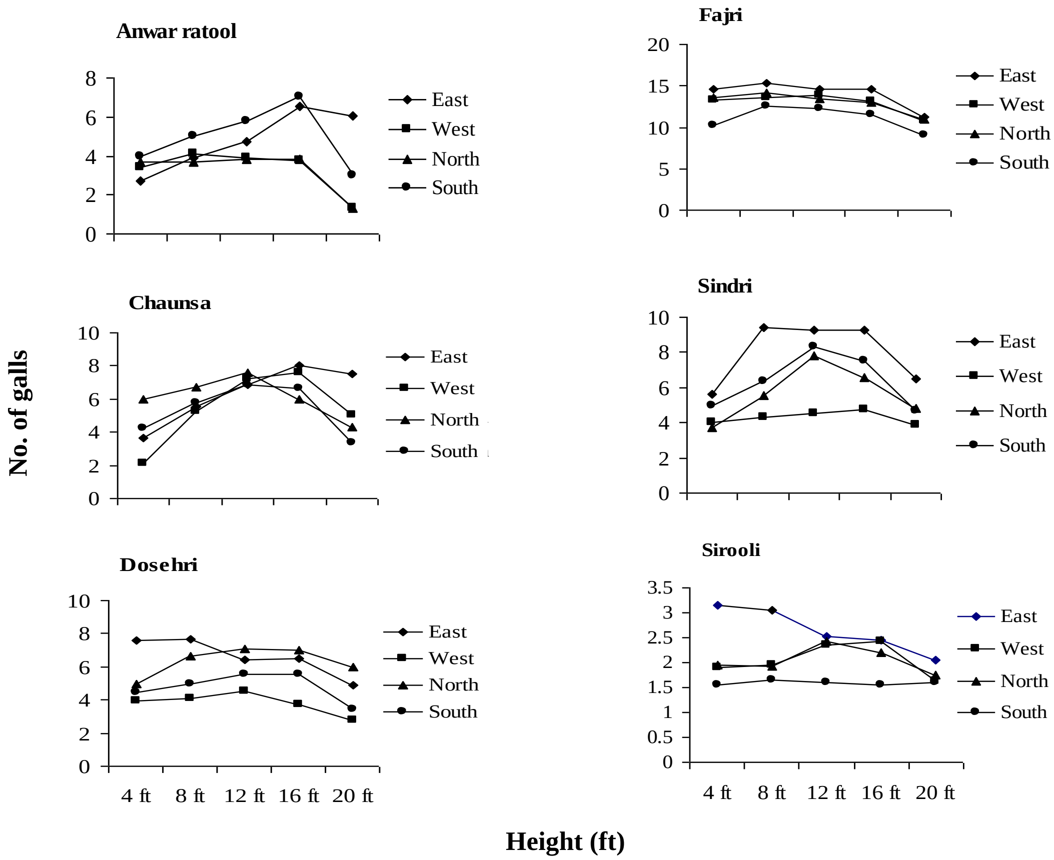

Figure 1. Mean no. of galls per five leaves in different quadrants and at different heights. The data were transformed to square root transformation $(X+0.5)^{1/2}$ before analysis.

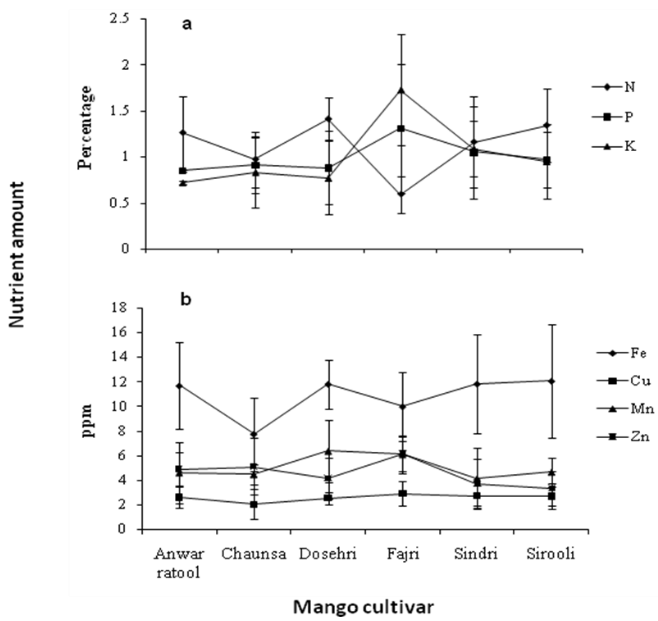

Figure 2. Amount of nutrients (mean ±SE) in the leaves of mango cultivars. a) percentage of nitrogen, phosphorus and potassium; b) parts per million (ppm) of iron, copper, manganese and zinc.

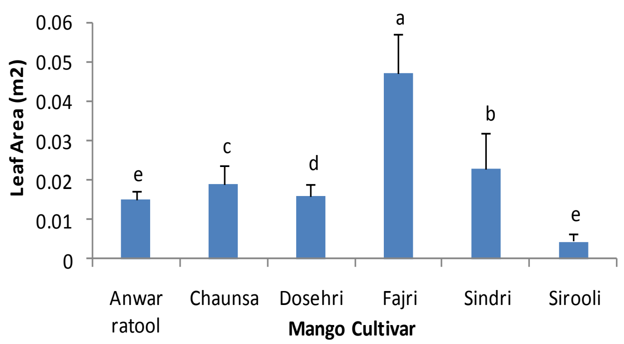

Figure 3. Bars are mean leaf area (m^2) (±SE). Bars topped with different letters are significantly different (P<0.01, $LSD_{0.05}$ test).

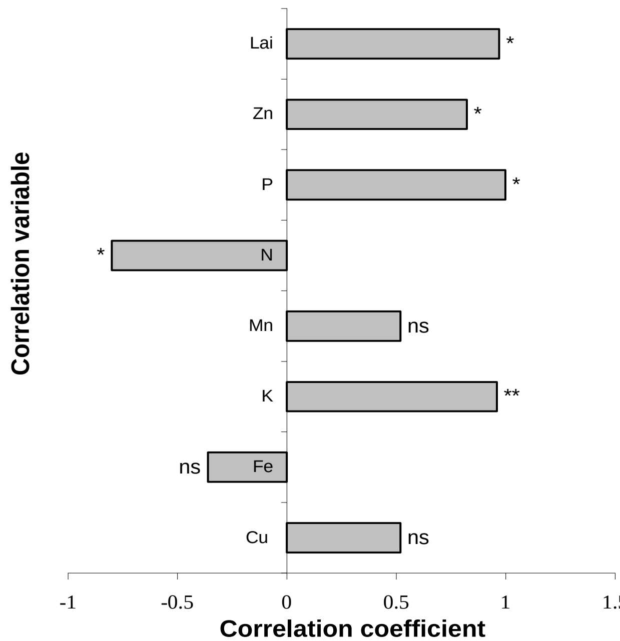

Figure 4. Bars are correlation coefficients (r) showing the extent of association between leaf parameters and abundance of galls in tested mango cultivars. La = leaf area, Zn = zinc, P = phosphorus, K = potassium, Fe = iron, Mn=manganese, N=nitrogen and Ca=calcium

to maximum (98.96%) when all the nutrients have been added to the model.

DISCUSSION

In the present study, the abundance of galls was variable on different mango cultivars and Fajri proved to be the most preferred cultivar by gall formers. One probable reason for this abundance might be due to difference in nutritional status of the leaves (Zafar *et al.*, 2010). Leaf samples of Fajri cultivar were found with minimum nitrogen, maximum phosphorus, potassium and zinc level and all these components showed a significant correlation with gall abundance (Fig. 4). The amount of nitrogen was found negatively correlated which means that the cultivars with a minimum level of nitrogen will have more gall abundance. Cultural practices, for instance fertilizer applications to crops could affect the insect-plant interactions by improving the plant tissue nutrient levels. Previously Altieri and Nicholls (2003) demonstrated that the chemical and biological properties of soils could adversely affect the pest infestation levels on a particular host plant.

Application of different types of nutrients to the soil has been considered to affect all the three categories of resistance proposed by Painter (1951): preference, antibiosis, and tolerance. The nutrient status of the soil not only affects the level of pest damage that plants receive from herbivores and/or disease pathogens but also the ability of plants to recover from injuries (Meyer, 1987). Of the nutrients, nitrogen has been considered an important candidate in attracting or deterring different pest to a particular host plant. (Slansky and Rodriguez, 1987; Aqueel and Leather, 2011). As plants are a nutritional source for herbivores, an increase in the nutrient content of the plant may be argued to increase its acceptance as a food source to pest populations (Altieri and Nicholls, 2003). Alterations in herbivore response may be explained by differences in the feeding behavior of the herbivores themselves. For instance, with increased level of nitrogen contents in creosotebush (*Larrea tridentate*) plants, the populations of sucking insect pests were found to increase, but the number of chewing insects turned down (Altieri and Nicholls, 2003). In this way, when Fajri cultivar had more abundant galls but had low nitrogen level and Sarooli had maximum nitrogen level (Fig. 3) but minimum gall abundance (Table 1), it might be possible that some chewing insects were contributing to gall formation. Insects from the orders of Diptera, Coleoptera, Hemiptera, Hymenoptera, Lepidoptera and Thysanoptera have the ability to induce plant galls. However, the shape or morphology of the galls depends on the type of causal organisms. For example, phylloxerans (Hemiptera) make blister galls and midges (Diptera) make spherical galls (Buss, 2008). So, there is a dire need to know about taxonomy of these gall formers particularly at Pakistan level. In the present study, intra tree distributional patterns were also an important phenomenon. The galls were more abundant at the heights of 12 ft and16 feet from the ground level and then declined. These findings are in accord as reported elsewhere (Anonymous, 2010) that the gall forming insects attack less above the height of 6 meters. The heterogeneous distribution of the pests within tree canopy is often reported (Pinero and Prokopy, 2005). Within the plant, physiological and chemical characteristics are not homogeneous: sectoriality in the allocation of resources

(Marquis, 1996) and induced plant defenses (Jones *et al.*, 1976) is widely documented. The complex process of within plant sectoriality is hypothesized to explain the different patterns of herbivory and to affect a wide range of categories of arthropod: chewers, suckers, borers and gall forming species (Orians and Jones, 2001). Moreover, east quadrant of mango trees was more prone to galler attack. The most probable reason for galler's preference in the east quadrant might be due to sunlight effects (Lebel *et al.*, 2008; Khan, 2010) as all the quadrants of a tree do not receive the same amount of light. However, further studies are needed to identify plant properties that may contribute to differences in distribution of gall formers within the mango plant.

The physicomorphic leaf characters may either have negative or positive influence on the pest population (Mattson, 1980). Different plant leaf characters like leaf area, leaf hair density, length of leaf, leaf thickness play a significant impact on the searching capability and the population of the natural enemies (Jones *et al.*, 1976). Leaf area, in the present study, was positively correlated with the gall abundance and maximum leaf area was found on the leaves of Fajri while the minimum was found of Sarooli cultivar.

In conclusion, there are several factors i.e., physico-morphic, the nutrient status of the leaves, and specific height of the plant, mediating preference for gall formers. This study provides an initiate about the behavior of gall formers influenced by different plant factors. However, there is a dire need to study the taxonomy of these gall formers particularly at Pakistan level, so that further studies on the management of these pests can be possible.

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