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Varietal and physico-morphic resistance of okra cultivars against *Amrasca biguttula biguttula* (Homoptera: Cicadellidae)

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Abstract
Effect of jassid population on different okra varieties was observed on the time frame at different life stages of the plant and effect of physio-morphic characters. Linear mixed models and growth curves were used by keeping plants effect random to account repeated measures data and cubic trend interaction with verities was found to be significant using AIC, BIC and deviance criteria of model selection. Nested designs were used for the area within each plant. Results revealed Arka Anamika (6.96 insects/plant) to be the most resistant cultivar among all the five tested cultivars followed by Super Green (8.11 isnects/plant). Ambika (13.3 insect/plant) was least resistant cultivar among all the five tested cultivars. Host plant resistance was enhanced by the hair density on plant leaves of okra cultivars; cultivars having high density of trichomes showed high resistance towards the jassid infestation. Leaf area differences drive the insect population was also calculated.

Keywords: Leaf area, jassid population, okra varieties, varietal resistance

Introduction
Okra (*Abelmoschus esculentus*) is cultivated worldwide and belongs to Malvaceae family. Okra is herbaceous annual plant of the old world tropics [14]. Its hairy leaves are lobbed and upto 11 inches in length and cultivars vary in pod color, shape and plant height and also vary in tolerance to rainfall [15]. Okra production is affected by number of bacteria, viruses, nematodes and insects and these losses upto 25% resulting income loss to growers.
Okra jassid, *Amrasca biguttula biguttula* (Homoptera: Cicadellidae) has become a serious pest of agronomic crops, vegetables and ornamental plants. It is the most destructive amongst sucking insect pests attacking the 'okra' crop [20]. Okra is the most suitable host for survival and feeding to its nymph [19]. The damage caused by *Amrasca. biguttula biguttula* starts from young seedling to the mature crop resulting in 50% yield loss [5]. Jassid' s attack causes the reduction in plant height and number of leaves up-to 49.8% and 45.1%, respectively [17].
Amrasca. biguttula biguttula is the most serious pest well distributed in Pakistan [10], India [7], Bangladesh, Thailand [8] and other Southeast Asian countries [2]. Excessive feeding damage the phloem tubes and causes disease, hopper burn (phytotoxemia) the main symptom of jassid attack [21]. Damage leaves develop brown and curl at the edges, stunted growth and unable to produce flowers and fruits and fall off leaves. Oviposition preference and subsequent population build-up on variety of host plants are tools for damage potential.
Host plant resistance is a major part of IPM program and is helpful when used with other control methods [13]. Resistant cultivars were less affected by jassid with respect to susceptible cultivars [11]. Density of trichomes on vein, subvein, and lamina have influenced on oviposition behavior [22]. Present studies were conducted to explore the role of host plant resistance and physico-morphic characters to *Amrasca biguttula biguttula* population.

Materials and Methods
The study was conducted on Physico-morphic resistance of different okra cultivars against *Amrasca biguttula biguttula* at University Farm, Koont of Pir Mehr Ali Shah Arid Agriculture University, and Rawalpindi during 2015. Five okra cultivars (Ambika, Arka Anamika, Rama Karishna, Sabaz Pari and Super Green) were selected.

Experimental layout

Experiment was laid out according to CRD with three replications.

The plot size was maintained up to 35 ft x 15 ft. The row to row and plant to plant distance was 2.5 ft and 2 ft, respectively. The seed was sown on parallel ridges. No plant protection measures were applied and material was screened under natural insect pressure. Normal agronomical practices were adopted to manage the crop. The data were recorded weekly basis early in the morning, starting three weeks after the sowing of crop. The data regarding nymph and adult population of *Amrasca biguttula biguttula* /leaf basis. Ten plants were selected randomly from each replication of each treatment and was examine in such a way that upper, middle and lower leaf of ten plants were observed with the help of magnifying glass and remaining examining of leaf was continued in the similar way to record the population of jassid. The population were calculated by applying the following formula

$$\text{Population (per leaf)} = \frac{\text{Total number of insects}}{\text{Number of leaves observed}}$$

To determine the physic-morphic characters of okra, following parameters were studied: Hair density/cm² on lamina, midrib and veins of the leaf was determine under microscope from three randomly selected leaves in each treatment. Five upper, middle and lower leaves were taken from each treatment. Leaf area was measured by using digital leaf area meter.

Statistical analysis

For Statistical analysis, growth curve models in lme4[3] package in R were used as it might possible to have a nonlinear pattern in time course data and different model selection criteria as AIC, BIC, log likelihood and deviance were employed [15]. So, higher order polynomials were used to account interaction of Okra varieties with higher orderd polynomials of time. For visualization of repeated measures data ggplot2 [23] package and for pairwise comparison of Okra varieties lsmeans function in lsmeans package in R was applied. To detect the significantly different areas of hair density among Okra varieties Turkeys test [1] was applied.

Results and Discussion

Five different varieties on the time frame against the jassid population were assessed for the insect damage. Varieties used in the experiment include Ambika, Arka Anamika, Rama Karishna, Sabz Pari, and Super Green. Figure 1 depict cubic trend among all five okra varieties over 18 weeks and showed an increasing trend in Jassid population. It also denote the continuous changes in the population of jassid throughout the data collection period. Population of jassid was varying in different varieties based on many factors responsible for preference or non-preference of the insect. Maximum population was recorded in Ambika variety during 17th week of data collection followed by the jassid population in Rama Karishna and Sabz Parik; both were at par. Minimum jassid population was recorded in Arka Anamika as shown in the figure 1.

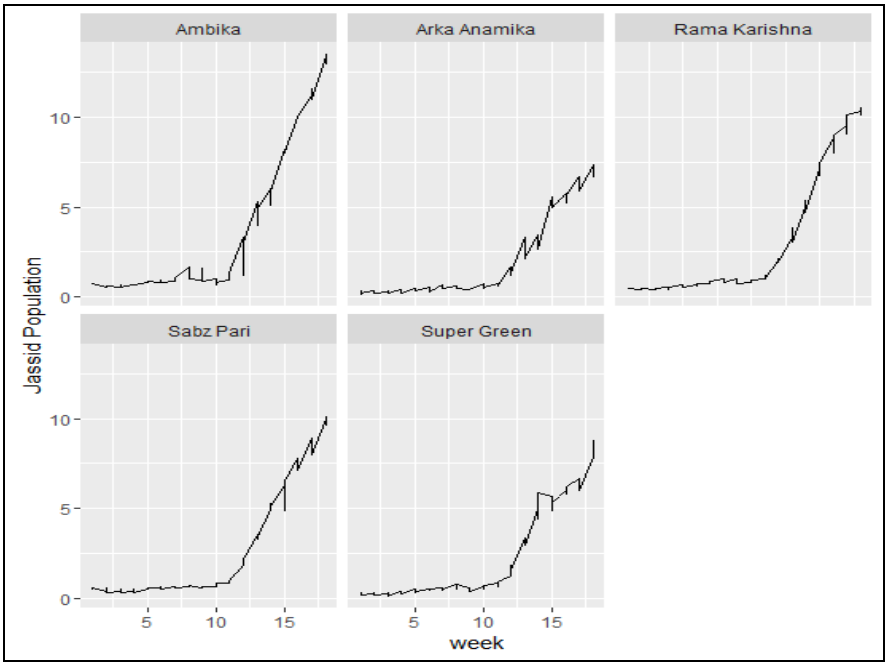

Fig 1: Facet plot of Varieties over weeks of Jassid population

Three different model were tested to check the integrity and effect of time frame and varietal differences on the jassid population. Models were as follow;

Linear interaction of weeks and varieties keeping plants as random effects.

Model 1

$$Jassid\ population = week + variety + week * Variety + (week|plants)$$

Model 2

$$\text{Jassid popualtion=week+week}^2 + \text{week}^3 + \text{variety+week}^3 * Variety + (week|plants)$$

Model 3

$$\text{Jassid popualtion=week+week}^2 + \text{week}^3 + variety + week * Variety + (week|plants)$$

AIC and BIC criteria was used to check goodness of model fit among 3 models. Model with minimum AIC and BIC was considered best. Also the p-value (<0.05) for Model2 shows significant imact of effects.

Table 1: Goodness of fit criteria for models

Model	Df	AIC	BIC	Log Lik	deviance	Chisq	Chi Df	Pr (>Chisq)
Model1	14	1055.923	1106.301	-513.962	1027.923			
Model2	16	484.7821	542.3568	-226.391	452.7821	575.1411	2	<0.0001
Model3	16	565.0111	622.5859	-266.506	533.0111	0	0	1

ANOVA was calculated for model 2 which is the best suited model of varieties against the jassid population over time frame and displayed below;

Table 2: ANOVA for Model 2

Source of variation	Df	Sum Sq	Mean Sq	F value
Week	1	1913.687	1913.687	5841.845
$Week^2$	1	580.5868	580.5868	1772.337
$Week^3$	1	17.29662	17.29662	52.8008
Variety	4	86.27717	21.56929	65.84384
$Week^3$*Variety	4	105.8997	26.47494	80.81912

Plot of observed (points) and fitted values (lines) of model 2 were shown in fig. 2 showing a cubic trend over time. Maximum jassid population in Ambika variety followed by Rama Karishna trailed by Sabz Pari. Jassid population in Arka Anamika variety calculated as minimum population of the jassid.

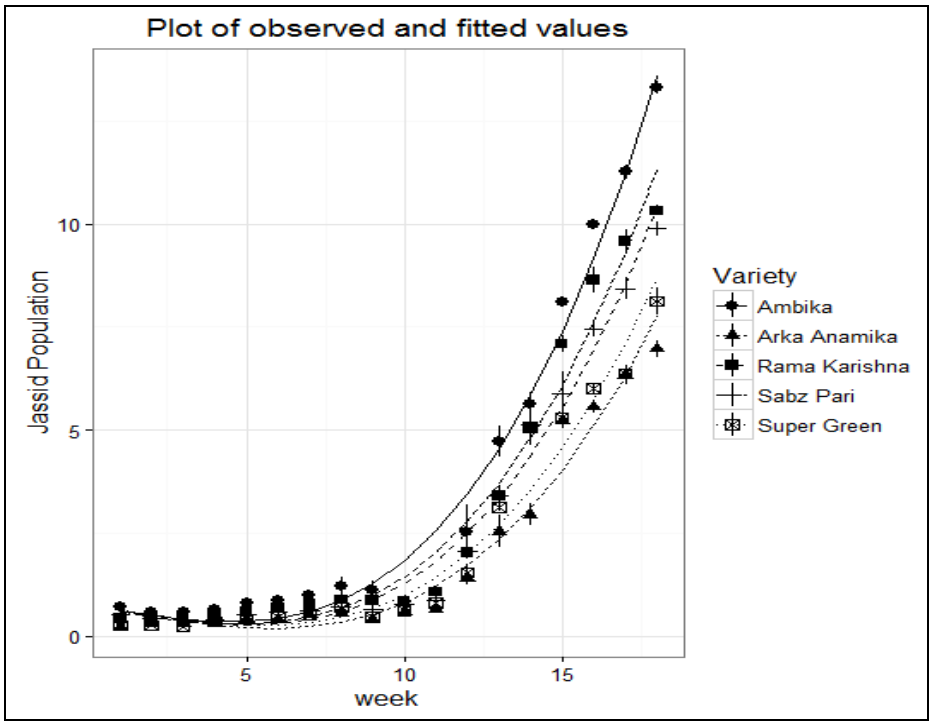

Table 3: Pairwise contrast analysis of Okra Varieties

	Contrast	Estimate	SE	Df	t.ratio	p.value
1	Ambika, Arka Anamika	0.876336	0.121926	10.46731	7.187462	0.00018*
2	Ambika, Rama Karishna	0.339799	0.121926	10.46731	2.786933	0.10569
3	Ambika, Sabz Pari	0.485866	0.121926	10.46731	3.984933	0.015881*
4	Ambika, Super Green	0.707866	0.121926	10.46731	5.805715	0.00107*
5	Arka Anamika, Rama Karishna	-0.53654	0.121926	10.46731	-4.40053	0.00831*
6	Arka Anamika, Sabz Pari	-0.39047	0.121926	10.46731	-3.20253	0.05503*
7	Arka Anamika, Super Green	-0.16847	0.121926	10.46731	-1.38175	0.65094
8	Rama Karishna, Sabz Pari	0.146067	0.121926	10.46731	1.197999	0.75274
9	Rama Karishna, Super Green	0.368067	0.121926	10.46731	3.018781	0.07359
10	Sabz Pari, Super Green	0.222	0.121926	10.46731	1.820782	0.41164

Pairwise contrast of different varieties with each other was done to check out the effect on each other and result revealed that contrast of |Ambika, Rama Karishna|, |Arka Anamika, Super Green|, |Rama Karishna, Sabz Pari| and |Sabz Pari, Super Green| were non-significant. Iqbal *et al.* [6] also found Arka Anamika to be the most resistant cultivar. Results of the present study were in accordance with the results of Shakeel *et al.* [18] who found Super Green to be moderately resistant cultivar among the tested okra cultivars.

Hair Density

Table 4: ANOVA for Hair Density in Different Varieties

Source of Variation	Df	SS	MS	F-value	P-Value
Variety	4	458	114.5	10.55	<0.0001
Area (Variety)	10	1064	106.4	9.80	<0.0001
Leaf Position (Area(Variaety))	30	10530	351.0	32.32	<0.0001
Residuals	90	977	10.9		

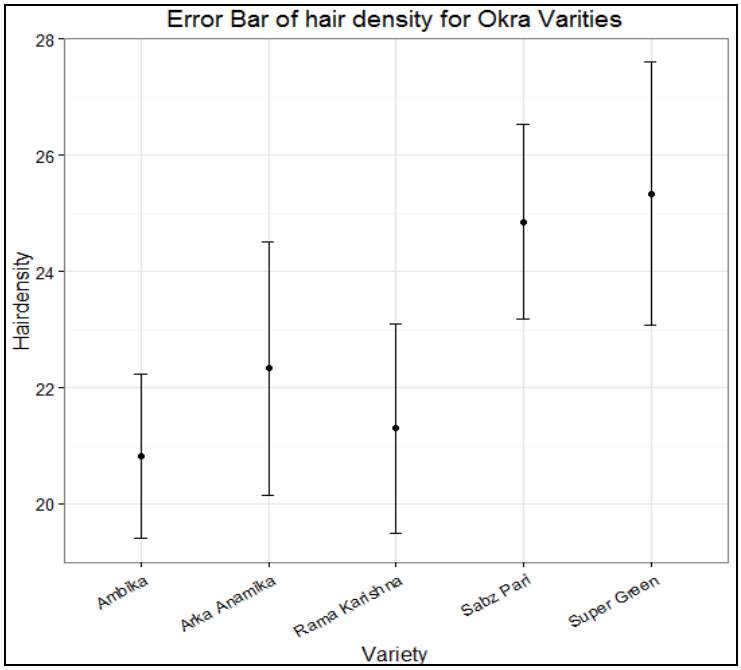

Fig 2: Hair Density Mean with error bars in Different Okra Varieties

Nested design is used for hair density data set as upper, middle and lower area is nested within a variety and leaf positions (Lemina, midrib, vein) is nested within area and then in each variety of okra. All the three sources of variation shows significant results towards the effect of hair density.

The results of the hair density showed that jassid has less preference to cultivars which had a higher hair density ratio and more preference towards the less dense leave hairs as Ambika is highly susceptible cultivar because of lowest hair density. Super green variety was the less resistive cultivar but has the highest hair density among other cultivars. Most resistant varieties based on hair density was Arka Anamika. But the comparison of cultivar Sabz Pari and Super Green against Arka Anamika cannot be done because jassid population was more in the first two varieties but unlikely have more hairs. So the results of the current study cannot be compared to the results of Mahal *et al,* [9] and Uthamasamy [22] who reported that hair density of the cultivar results in more host plant resistance towards the sucking insect pest of okra.

Leaf area difference among different varieties were calculated. Varieties having significant different among leaf area among different varieties have been shown in Table 4. Many non-significant response have been received. But only significant result are shown in the table.

Table 5: Area within each variety of Okra

	Difference	Lower	Upper	p adjusted
Super Green: Middle-Ambika: Lower	6.556	1.138	11.973	0.0049
Ambika: Upper-Ambika: Lower	5.667	0.249	11.084	0.0313
Sabz Pari: Upper-Ambika: Lower	9.444	4.027	14.862	0.0000
Super Green: Upper-Ambika: Lower	10.667	5.249	16.084	0.0000
Rama Karishna: Lower-Arka Anamika: Lower	-5.556	-10.973	-0.138	0.0387
Sabz Pari: Upper-Arka Anamika: Lower	6.111	0.693	11.529	0.0128
Super Green: Upper-Arka Anamika: Lower	7.333	1.916	12.751	0.0008
Sabz Pari: Lower-Rama Karishna: Lower	5.778	0.360	11.195	0.0252
Rama Karishna: Middle-Rama Karishna: Lower	5.778	0.360	11.195	0.0252
Sabz Pari: Middle-Rama Karishna: Lower	6.444	1.027	11.862	0.0063
Super Green: Middle-Rama Karishna: Lower	8.778	3.360	14.195	0.0000
Ambika: Upper-Rama Karishna: Lower	7.889	2.471	13.307	0.0002
Arka Anamika: Upper-Rama Karishna: Lower	5.889	0.471	11.307	0.0202
Rama Karishna: Upper-Rama Karishna: Lower	7.444	2.027	12.862	0.0006

Sabz Pari: Upper-Rama Karishna: Lower	11.667	6.249	17.084	0.0000
Super Green: Upper-Rama Karishna: Lower	12.889	7.471	18.307	0.0000
Sabz Pari: Upper-Sabz Pari: Lower	5.889	0.471	11.307	0.0202
Super Green: Upper-Sabz Pari: Lower	7.111	1.693	12.529	0.0013
Sabz Pari: Upper-Super Green: Lower	8.000	2.582	13.418	0.0001
Super Green: Upper-Super Green: Lower	9.222	3.805	14.640	0.0000
Super Green: Middle-Ambika: Middle	7.111	1.693	12.529	0.0013
Ambika: Upper-Ambika: Middle	6.222	0.805	11.640	0.0101
Rama Karishna: Upper-Ambika: Middle	5.778	0.360	11.195	0.0252
Sabz Pari: Upper-Ambika: Middle	10.000	4.582	15.418	0.0000
Super Green: Upper-Ambika: Middle	11.222	5.805	16.640	0.0000
Sabz Pari: Upper-Arka Anamika: Middle	6.778	1.360	12.195	0.0030
Super Green: Upper-Arka Anamika:Middle	8.000	2.582	13.418	0.0001
Sabz Pari: Upper-Rama Karishna: Middle	5.889	0.471	11.307	0.0202
Super Green:Upper-Rama Karishna: Middle	7.111	1.693	12.529	0.0013
Super Green: Upper-Sabz Pari:Middle	6.444	1.027	11.862	0.0063
Sabz Pari: Upper-Arka Anamika: Upper	5.778	0.360	11.195	0.0252
Super Green: Upper-Arka Anamika: Upper	7.000	1.582	12.418	0.0018
Super Green: Upper-Rama Karishna: Upper	5.444	0.027	10.862	0.0476

The results of the infested and un infested leaves were in accordance with the results of Mandal *et al,* [12] and Bhat *et al.* [4] who also found that the infested leaves had low vigor with respect to the non-infested leaves.

Conclusion

It is evident from the current study that variety Arka Amika was most resistant varieties and variety Ambika was most susceptible one based on the *Amrasca biguttula biguttula* population. Interaction of the pest population with hair density was negative and leaf area interaction to the population of jassid calculated positive.

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