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Effect of xenobiotics on adult banana pseudo stem weevil odoiporus longicollis oliv. (Coleoptera: Curculionidae), using landmark based geometric morphometric analysis

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Abstract
The effects of xenobiotic on the adult banana pseudo stem weevil were investigated using the geometric morphometric method. The study examined the symmetry and compared wing patterns of *Odoiporus longicollis* collected from two selected sites such as pesticide treated Vellalassery area and pesticide untreated Chathamangalam area in Calicut District, Kerala. In this study total 18 landmarks were identified and marked on the hind wing. Procrustes superimposition analysis of geometric morphometric is performed where, only the shape information is considered. The cumulative percentage of variation of first three principal components was 62.163%. The Canonical Variate Analysis (CVA) results showed that the different groups of male and female insect exhibit variation and asymmetry in hind wing, as the Maha lanobis and Pro crustes distances for each group are significantly different (p<.0001). Based on the PCA and CVA studies it is very clear that there exists great variation in hind wing size and shape among and between the control and contaminated groups.

Keywords: xenobiotics, banana pseudo stem weevil, geometric morphometric, shape analysis, CVA, PCA

Introduction
Taxonomic classification and understanding of biological diversity have been based mainly on morphological descriptions [1] but recently, comparative biology entered a transition from the description field and quantitative science, where morphological analysis had a similar revolution of quantification [2] which made possible the combination of multivariate statistical methods and new ways to visualize a structure [3, 4]. Shape analysis is one of the statistical methods for understanding morphological variability and shape as all the geometric features of an object except its size, position and orientation and due to the advancement in statistics, geometry and biology analysis of shape has become more quantitatively described, which lead to the development of geometric morphometrics; the fast and reliable way of studying biological forms [5]. Land mark based geometric morphometric are used mostly for the study of sexual dimorphism in organisms where homologous points in biological structures are studied and also is an important source for phenotypic variation in organisms. It is also described that landmarks as points that can be located precisely on each specimen under study with a clear correspondence in a one-to-one manner from specimen to specimen [6,7].

Coleoptera (known as beetles) are the largest insect order, containing 3.80.000 named living species classified into more than 160 families [8]. The body of coleopterans is covered by tough exoskeleton which protects its membranous body parts. The fore wing is modified into hardened elytra which are usually not used for flight. It serves as a protective cover over hind wing and hind wing is membranous and folded under the elytra [9]. Hind wings are folded longitudinally and transversely under elytra. The hind wing must have a certain size to be aerodynamically functional, which makes them distinctly larger than the thickened fore wings [10]. Studies on the wing folding mechanisms of beetles by Beutel and Haas [11] explains that longitudinal folding was already present in the earliest stem-lineage representatives of the Lower Permian, whereas transverse folding evolved in the Middle Permian with the formation of a closed sub elytral space and the hind wings are unfolded during flight [12]. Morphological variances of insect wings have been done using geometric morphometric analysis [13,14.15] and using the geometric morphometric, analysis of insect wings has applied in insect taxonomy [16,17]. The present study aims to determine the effect of xenobiotics on adult banana pseudo stem weevil, Odoiporus longicollis using landmark based geometric morphometric analysis.

Materials and Methods
The adult banana stem weevil, Odoiporus longicollis were collected from two selected sites such as pesticide treated Vella lassery area (contaminated) and a pesticide untreated Chathamangalam area (uncontaminated) in Calicut District, Kerala during January to June 2017. A total of 94 insects collected from the banana plantations treated with chemical pesticides. Of the 94 insects collected, 46 were males and 48 were females. Another group of 110 adult banana weevils were collected from banana plantations in the Chathamangalam Panchayath in Kozhikode district where only organic fertilizers were used. Of these 59 were males and 51 were females. The adult banana weevil exhibits sexual dimorphism and the male and female were identified based on their rostral surface. After identification, the adult insects were killed and hind wings were carefully removed using forceps. Insect wings were separated and photographed with a scale bar included to record the size of each specimen. The statistical differentiation was performed by one way ANOVA and size variation by centroid size.

Landmarks
For the morphological analysis, 18 landmarks for hind wings of banana weevil were identified (table 1). The

locations of landmarks were chosen based on the intersection of veins or vein base, vein end or apex. The “anterior”, “posterior”, “proximal”, and “distal” veins or plates were used to describe the detailed position of landmarks (plate 1). Landmarks are defined as points of correspondence on each object that matches between and within populations [4]. Wings were dissected from anesthetized specimens and documented by using CANON EOS 7 D camera.

Landmarks for geometric morphometric analysis were digitized using tips. dig software. Analysis and interpretation are done using Morph5 software using principal component analysis and canonical variate analysis. Morpho J (Version 1.06d), software package enabling geometric morphometric analysis for two-dimentional and three-dimensional landmark data and designed for the analysis of actual biological data is used for landmark data analyses [18]. Prior to further analyses, the landmark data of wings were imported into Morpho J, and a complete Procrustes fit was conducted by orthogonal projection to correct size and orientation. The digitized images were superimposed by generalized procrustes superimposition. The position description of landmarks and the average shape of hind wing of weevil is provided in table 1.

Table 1: Landmark position description, mean configuration of the 18 landmarks plotted of the wing of banana weevil.

Landmark	Descriptive location	Description	Average shape	
			Axis 1 (x)	Axis 2 (y)
1	sc(b)	basal end of the sub costa	0.212183	0.040238
2	sc(d)	distal end of the sub costa	0.066874	0.067889
3	hi	anterior hinge	-0.01495	0.073999
4	r(b)	basal end of anterial radian	0.221102	0.026861
5	ra 3 (b)	basal end of the anterial radius$_3$	-0.01013	0.053726
6	ra$_3$(d)	distal end of anterial radius $_3$	-0.40523	0.084442
7	ra$_4$+rp$_1$(b)	basal end of anterial radius$_4$+ posterior radius $_1$	0.001336	0.035049
8	ra$_4$+rp$_1$(d)	distal end of anterial radius$_4$+ posterior radius $_1$	-0.50031	0.030826
9	rp$_2$(b)	basal end of radius postrior$_2$	0.01428	0.013527
10	rp$_2$(d)	distal end of radius postrior$_2$	-0.39773	-0.06543
11	mp$_{1+2}$(b)	basal end of posterio median$_{1+2}$	0.236769	0.015323
12	mp$_{1+2}$(d)	distal end of posterio median$_{1+2}$	-0.00354	-0.02094
13	rp$_{3+4}$(b)	basal end of radius posterior$_{3+4}$	-0.01789	-0.01254
14	rp$_{3+4}$(d)	distal end of radius posterior$_{3+4}$	-0.15329	-0.12886
15	cua(b)	basal end of cubito anal	0.264069	0.010233
16	cua(d)	distal end of cubito anal	0.047532	-0.13545
17	aa(b)	basal end of anterior anal	0.275997	0.004872
18	aa(d)	distal end of anterior anal	0.162914	-0.09377

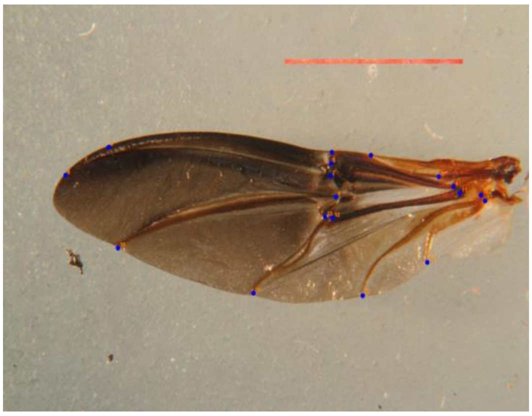


Plate 1: Digitalized image of hind wing of *Odoiporus longicollis* with landmark points**.**

Principal Component Analysis (PCA)

PCA is the most widely used method for exploratory multivariate analysis [19, 13], for perceiving wing shape variation between sexes, visualized by using deformation grid and discriminant analysis. In this study, Morpho J generates covariance matrices from landmark data sets of 200 specimens after Procrustes superimposition (table 2). Based on the covariance matrices, PCA is used to analyse and display the patterns of cova rations of positions of landmarks throughout the wing. Principal components (PCs) are visualised directly as patterns of simultaneous displacements of landmarks in relation to one another. The data set contains 690 observations of which 664 are included for analyses as groups of UC, F (uncontaminated, females-190 observations), CM (contaminated, males 100-observations), CF (contaminated, females-193 observations) and CF (contaminated, females -181 observations).

Canonical Variate Analysis (CVA)

CVA is a method used to find the shape features that best distinguish among multiple groups of specimens [20, 14]. CVA based on 200 specimens are used to explore the wing variance of male and female weevils collected from contaminated and uncontaminated area.

Results

On the basis of analysis of hind wing of banana weevil, *Odoiporus longicollis,* the data obtained showed significant variation in size and shape of hind wings of two sexes. The Procrustes analysis were performed on the acquired landmarks data of wings to determine the average shape within the subgroup of adult male and female beetles and also among and between both contaminated and uncontaminated area (table 2,3). The landmarks are then superimposed to optimize the distances from a common centroid shape. The outcome of this study provides detailed information on the average shape and variation in wing morphology between male and female adult beetles *Odoiporus longicollis* and data were graphically represented. Using the canonical variate analysis (CVA) and principal component analysis (PCA), the symmetry between left and right hind wings of male and female adult insects were analyzed in pesticide free and pesticide affected group and graphically represented it. Also, the wing shape variations caused by the pesticides on *Odoiporus longicollis* is also represented in shape decomposition grid for the two groups.

Table 2: Statistical analysis showing significant difference between Contaminated and Uncontaminated groups

Mahalanobis distances among groups				**P-values from permutation tests**			
	UC, F	UC, M	C, F		UC, F	UC, M	C, F
UC,M	1.4908			UC, M	<.0001		
C,F	3.8303	4.1653		C, F	<.0001	<.0001	
C,M	3.3349	3.5624	1.4727	C, M	<.0001	<.0001	<.0001
Procrustes distances among groups:				P-values from permutation tests			
	UC, F	UC, M	C, F		UC, F	UC, M	C, F
UC,M	0.0196			UC,M	0.0004		
C,F	0.0371	0.0389		C,F	<.0001	<.0001	
C,M	0.0437	0.041	0.0191	C,M	<.0001	<.0001	<.0001

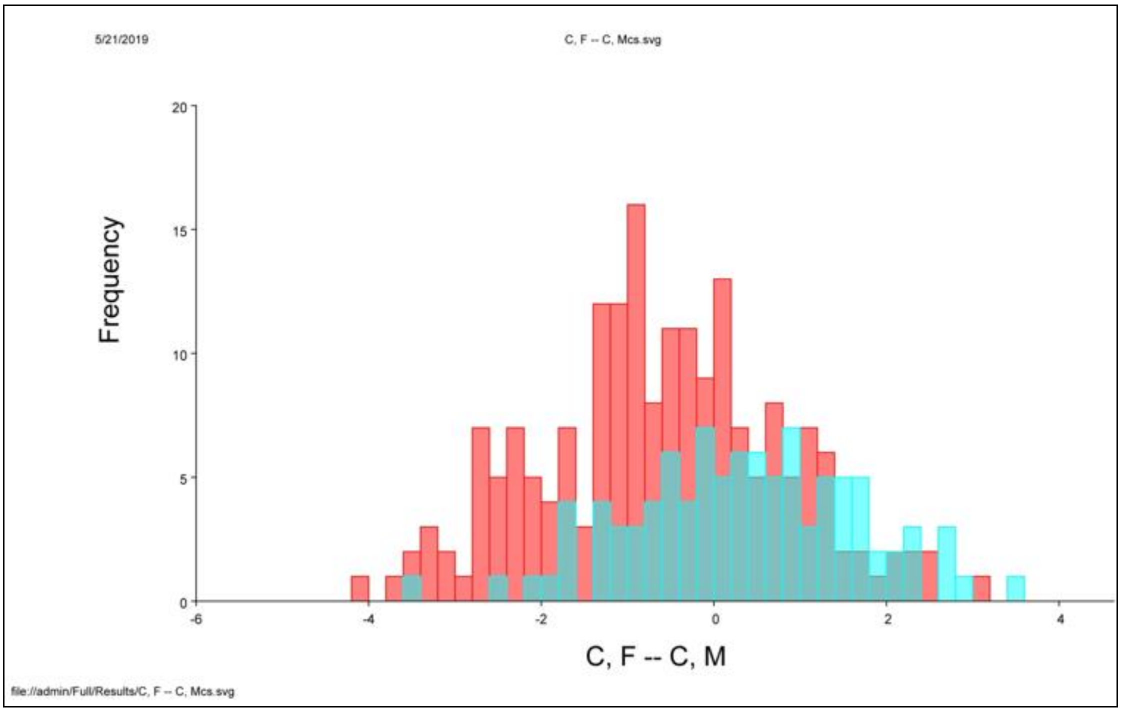
Table 3: Procrustes analysis of variance (ANOVA) and decomposition of shape

Centroid size: Procustes ANOVA		**Hind wings uncontaminated and contaminated**			
Effect	Sum of squares	Mean square	dF	F	P (param.)
Individual	50930039.755611	1083617.867141	47	1.18	0.2862
Side	171469678.781694	171469678.781694	1	186.75	<.0001
Ind * Side	43154622.414642	918183.455631	47	0.14	1.0000
Error 1	659849487840002	6665146.341818	99	3.96	<.0001
residual	720107583.824735	1682494.354731	428		
Shape: Procustes ANOVA		Hind wings uncontaminated and contaminated			
Effect	Sum of squares	Mean square	dF	F	P (param.)
Individual	0.24462334	0.0001626485	1504	1.02	0.3682
Side	0.21390323	0.0066844758	32	41.82	<.0001
Ind * Side	0.24041356	0.0001598494	1504	1.55	<.0001
Error 1	0.32765134	0.0001034253	3168	1.22	<.0001
residual	1.16214470	0.0000848529	13696		

In principal component analysis, the first principal component has the highest variation. The variations decrease with each component. The cumulative percentage of variation of first three principal components was 62.163%. The variation percentages of PC1, PC2, and PC3 were 36.882, 18.122, and 7.159 respectively. The results show the asymmetry between uncontaminated and contaminated groups. The significant P-value (<0.0001) obtained in left and right hind wing analysis shows the variation and asymmetry between the pesticide free and pesticide treated groups.

Male and female hind wing size and shape variations in insects collected from uncontaminated area.

Among this pesticide free area group, the male and female Showed significant (<.0001) variations indicating the wing asymmetry. The differences in hind wing shape of male *O. longicollis* in control and experimental conditions were compared and visualized through graphs. Using the thin plate deformation grid the wing shape differences between (pesticide free area) control and contaminated (pesticide treated area) individuals were shown. The male and female insects showed distortions in the wing which is represented in the graph. The land marks that showed maximum deviations are at the distal regions of the wing. They are distal end of radius posterior$_{3+4}$ (RP_{3+4} (D)), distal end of anterial radius $_3$ (RA_3(D)), distal end of anterial radius$_4$+ posterior radius $_1$(*in vitro* RA_4+RP_1(D)) (Fig1).



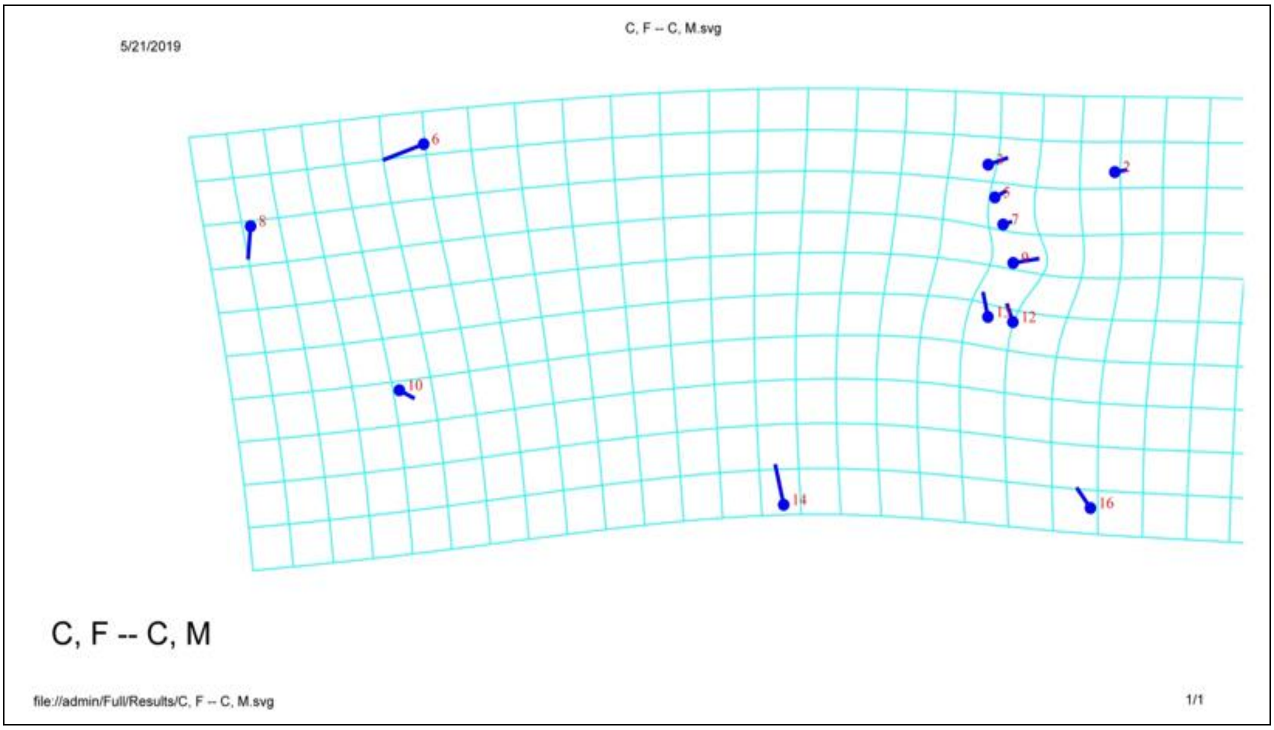
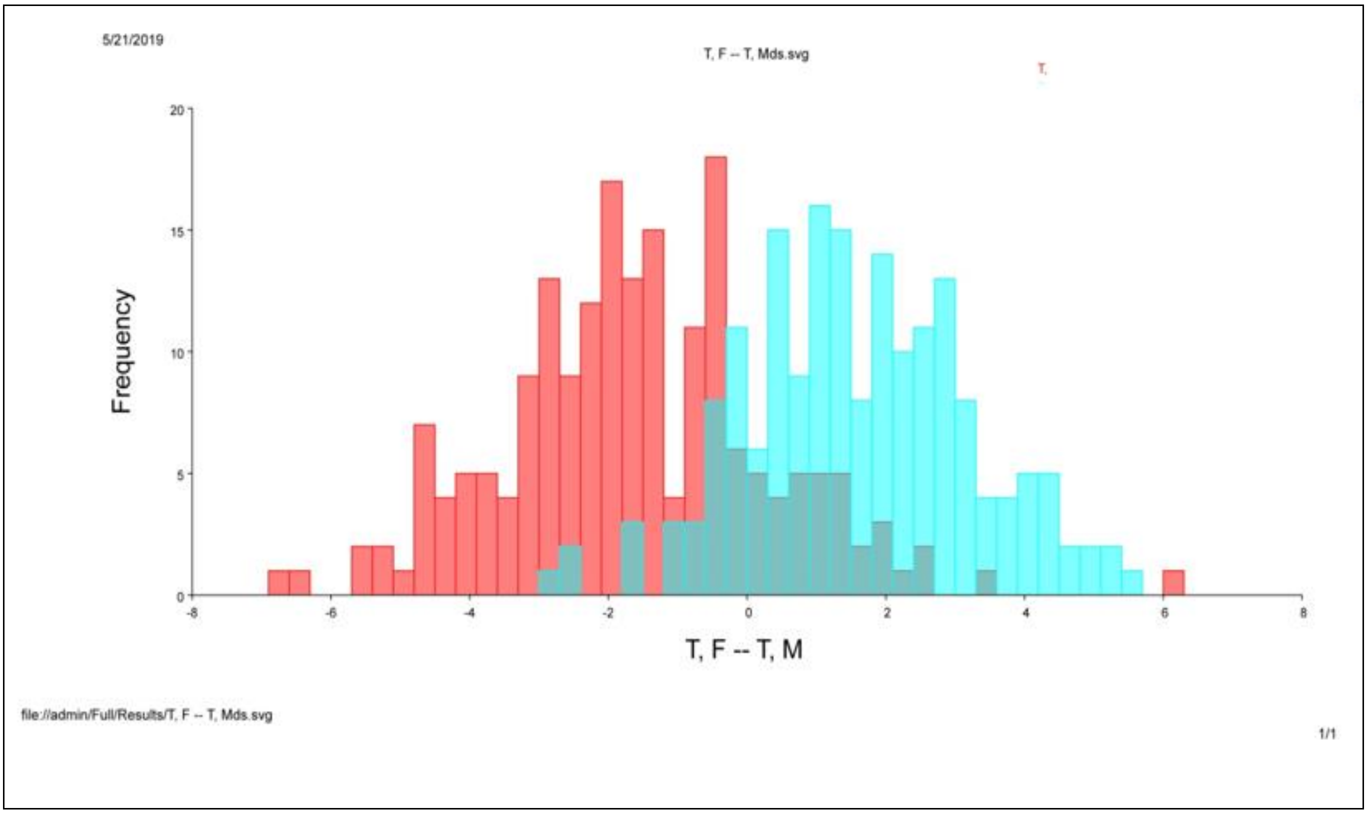


Fig 1: (a)- Discriminate analysis data showing variation in male and female hind wings of insects of pesticide free area, (b)-Thin plate deformation grid representing insects from pesticide free area hind wings of both sexes.

Male and female hind wing size and shape variations in insect collected from contaminated area

The insects collected from the pesticide treated area shows a high variation in size and shape of the hind wing. The P-value obtained (<.0001) showed significant variation in size and shape. The land mark point six showed highest variation in position compared to others. Distal end of anterial radius3, RA_3 (D) is the land mark at this position. Landmark number 18 (AA (D)) and 4(R (B)) (Fig. 2) showed no deviation in position. This implies that the position of these landmarks is almost similar in the case of forewings of contaminated male and female insects.



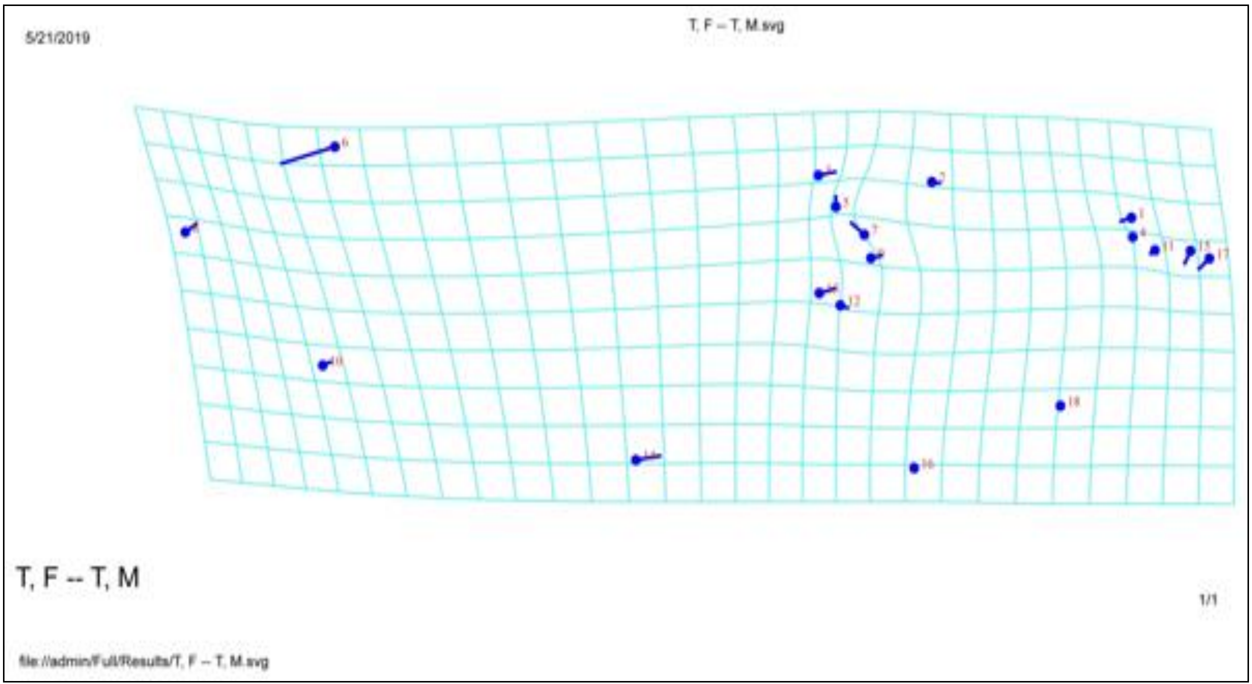
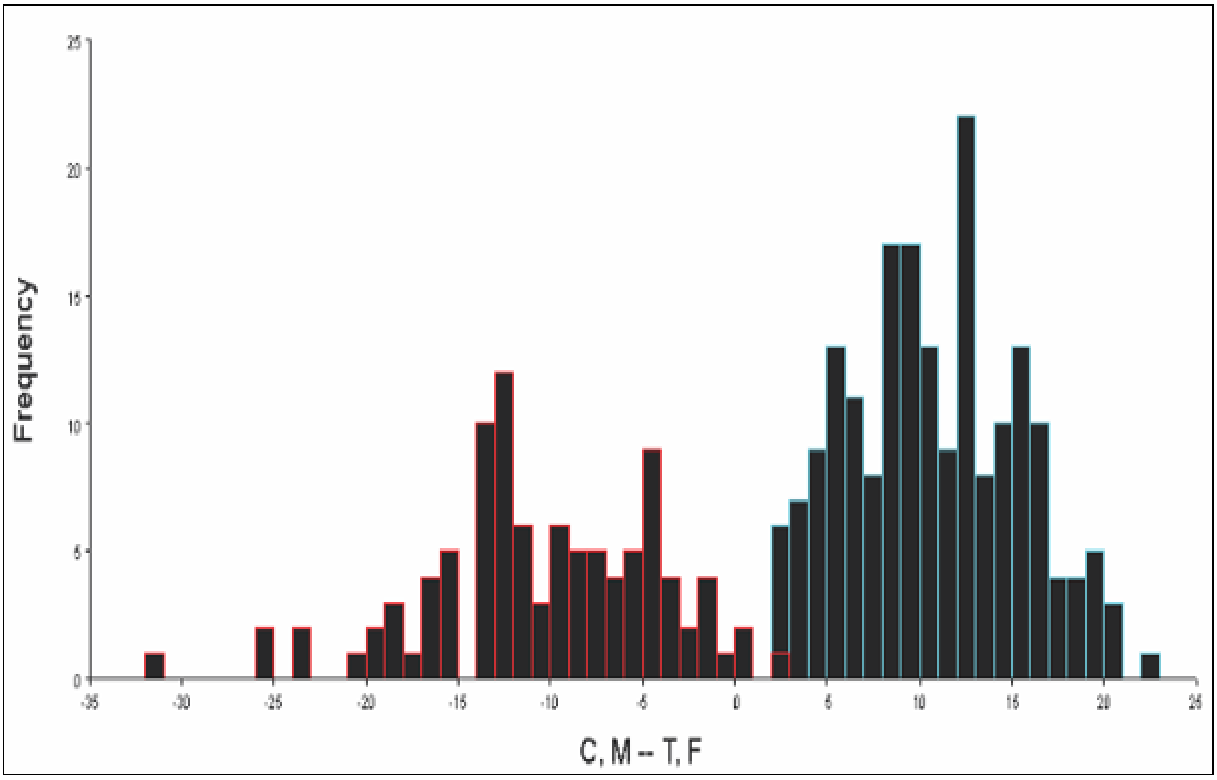


Fig 2: (a) Discriminant analysis representing hind wings of male and female Insects from pesticide treated area. (b)-Thin plate deformation grid representing treated insects' hind wings of both sexes

Uncontaminated Male and Contaminated Female Hind Wing Size and Shape Variations

The discriminant analysis graph revealed that most of the landmark points in the forewing showed high deviation. The complete separation between male and female in the graph denotes the shape deterioration of individuals of male and female of control and contaminated area. Here also, there is a significant variation of hind wing size and shape (<.0001). Distal end of the sub costa, anterior hinge, basal end of anterial radian, basal end of the anterial radius$_3$, distal end of anterial radius $_3$, basal end of anterial radius$_4$+ posterior radius $_1$,distal end of anterial radius$_4$+ posterior radius $_1$,basal end of radius posterior$_2$, distal end of radius posterior $_2$, basal end of posterio median$_{1+2}$,distal end of posterio median$_{1+2}$,basal end of radius posterior$_{3+4}$,distal end of radius posterior $_{3+4}$,basal end of cubito anal, distal end of cubito anal, basal end of anterior anal are the points of deviations. The thin plate deformation grid shows the wing shape differences between treated and untreated insects (Fig 3).



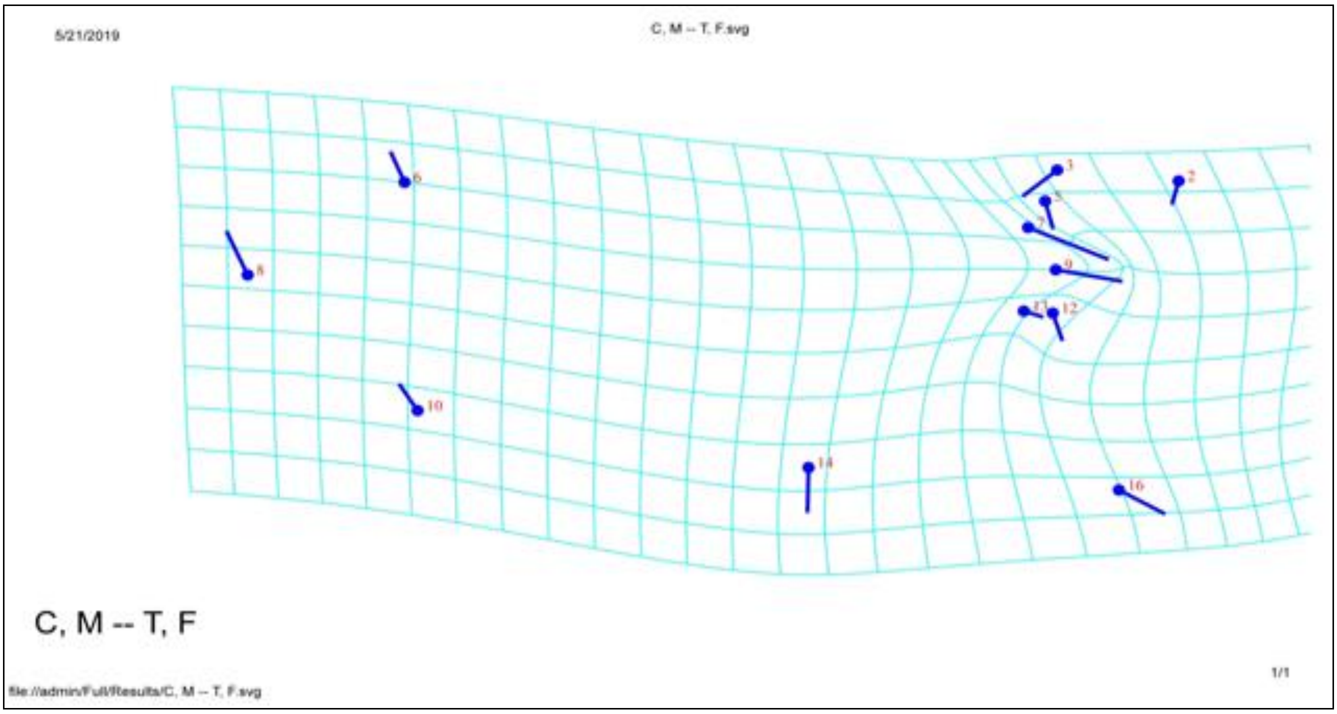
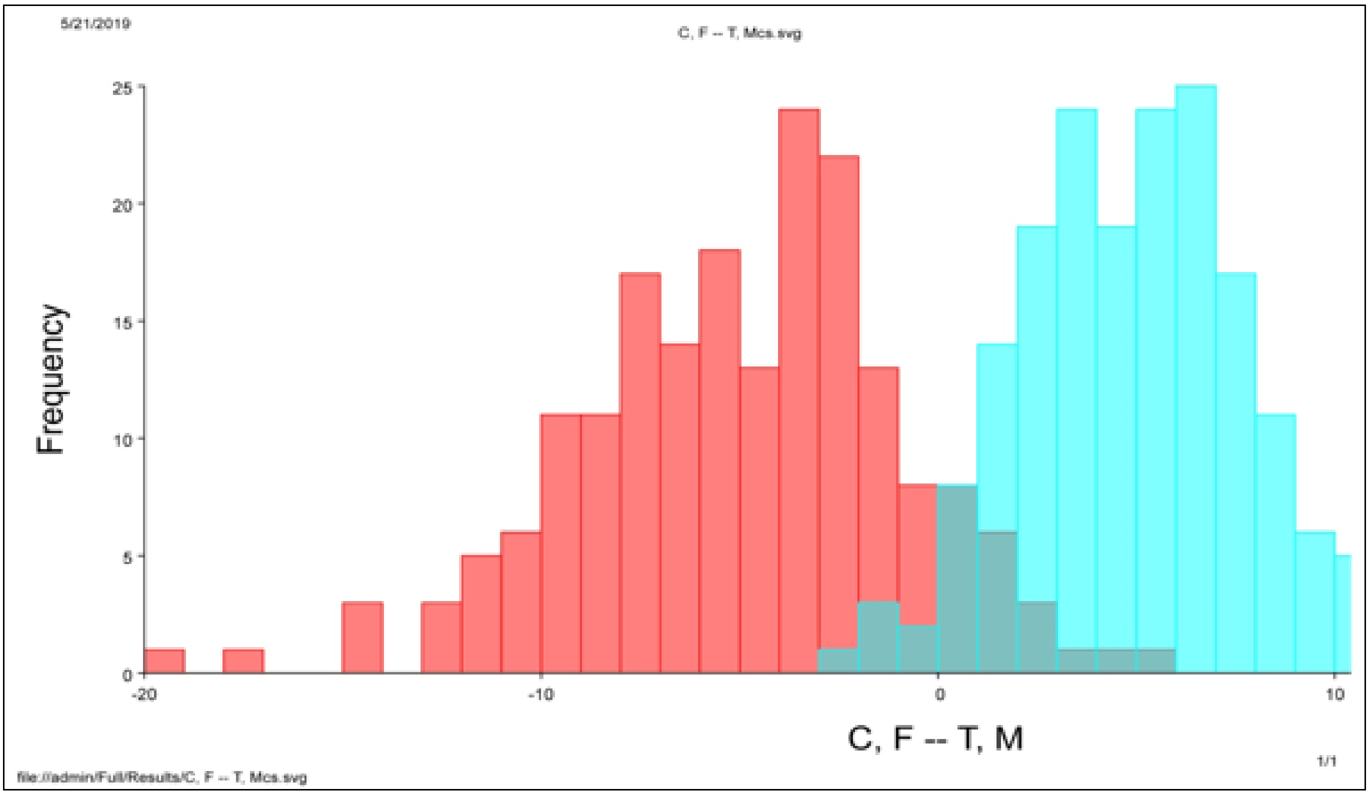


Fig 3: (a) - Discriminate analysis data showing variation in control male and female from contaminated area. (b) -Thin plate deformation grid representing treated insect's hind wings of female and control male

Uncontaminated Female and Contaminated Male Hind Wing Size and Shape Variations

The difference in the hind wing shape and size between control and contaminated insects were compared and visualized through discriminant analysis and thin plate deformation grid. distal end of anterial radius $_3$, basal end of radius posterior$_2$, basal end of anterial radius$_4$+ posterior radius $_1$, basal end of anterior anal, basal end of cubito anal, basal end of posterio median$_{1+2}$, basal end of anterial radian and basal end of radius posterior$_{3+4}$ showed deviations. The highest variation is observed in distal end of anterial radius $_3$. Significant P value obtained in forewing shape of female (<0.0001) indicated asymmetry between undisturbed and disturbed individuals (Fig 4).



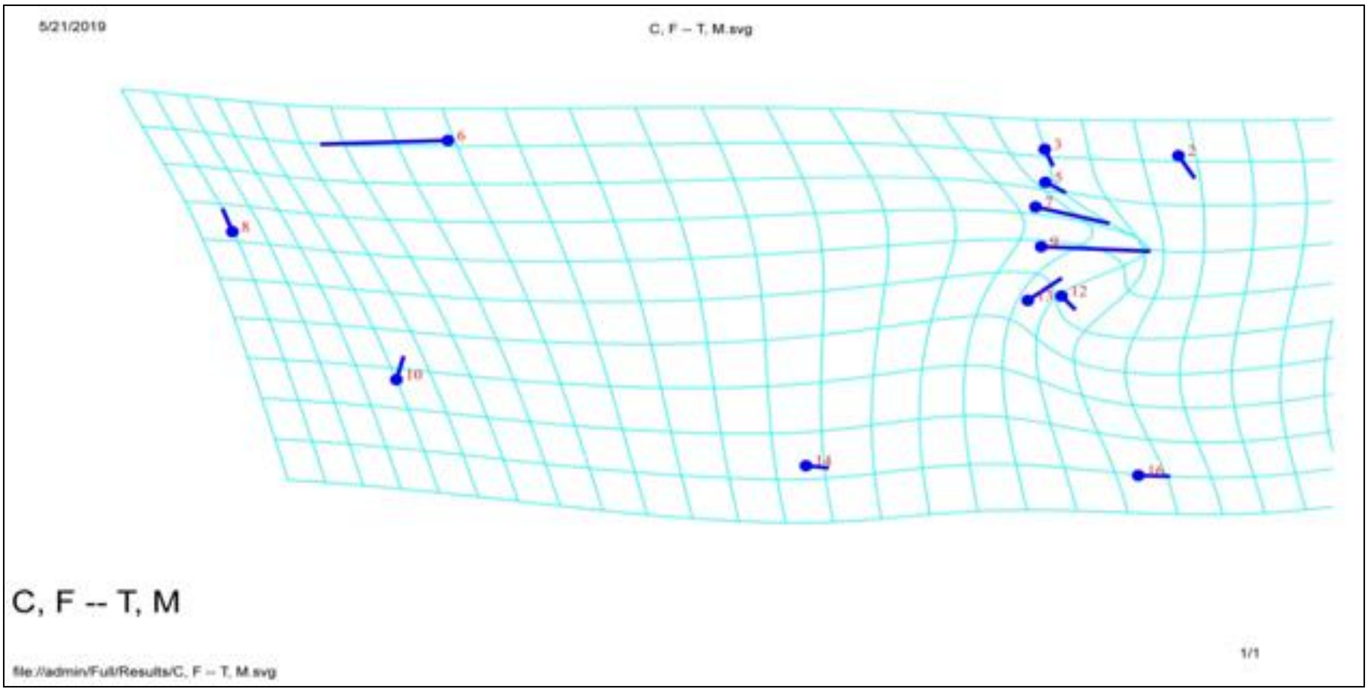
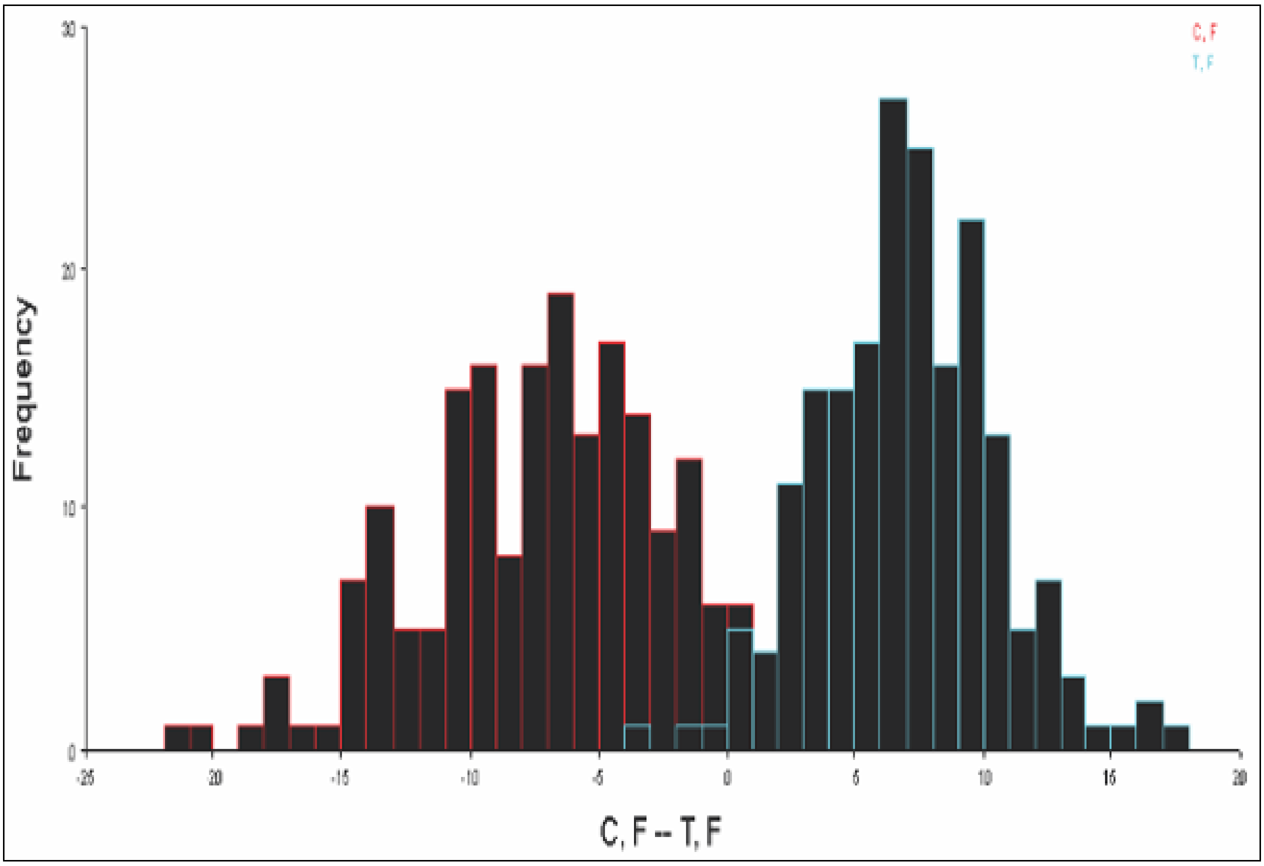


Fig 4: (a)-Discriminant analysis representing hind wings of control female and male from insects from contaminated area. (b)-Thin plate deformation grid representing treated insect's hind wings of male and control female.

Uncontaminated and Contaminated Female Hind Wing Size and Shape Variations

The deviation in hind wing shape of the control female from contaminated females is shown in the graph. The thin plate deformation grid of the forewings of both treated and untreated females exhibited deviation in almost all landmarks points. The minimum deviation was observed in landmark number 18 (distal end of anterior anal) and maximum deviations were seen in landmark number 7 [RA_4+RP_1(B)], 9 [RP_2(B)],15 [CuA(B)],17 [AA(B)],6 [RA_3(D)]. The highly significant P value (<.0001) indicates the difference in the size and shape of the insect hind wings (Fig 5).



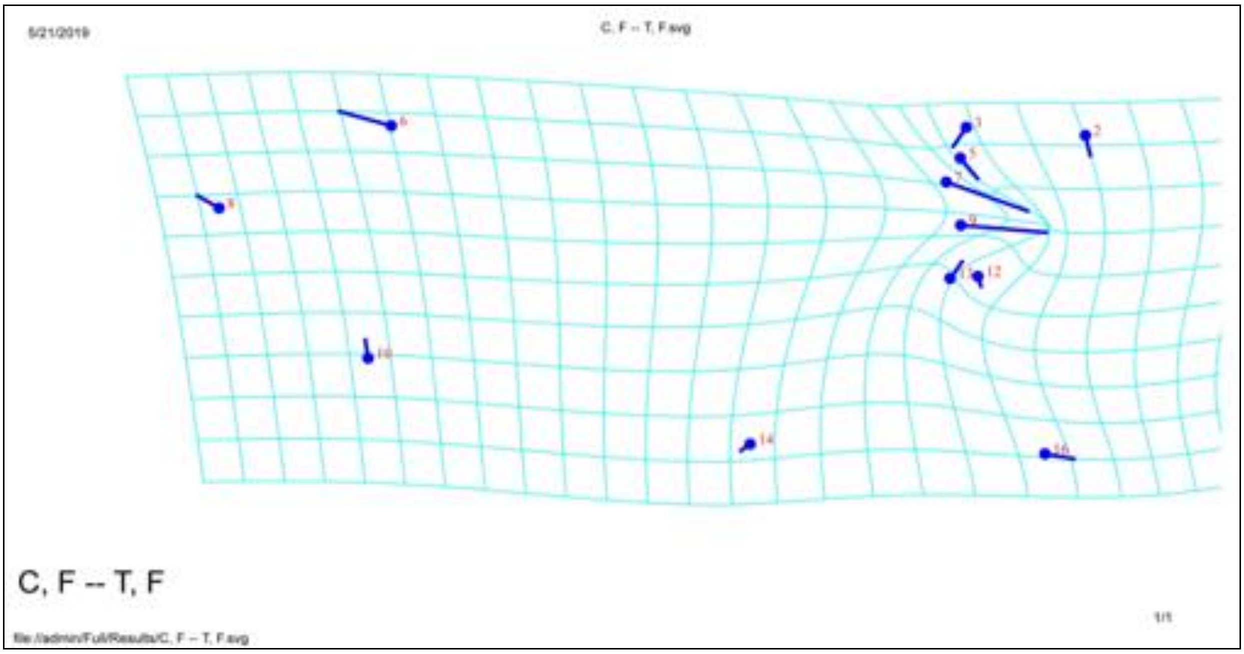
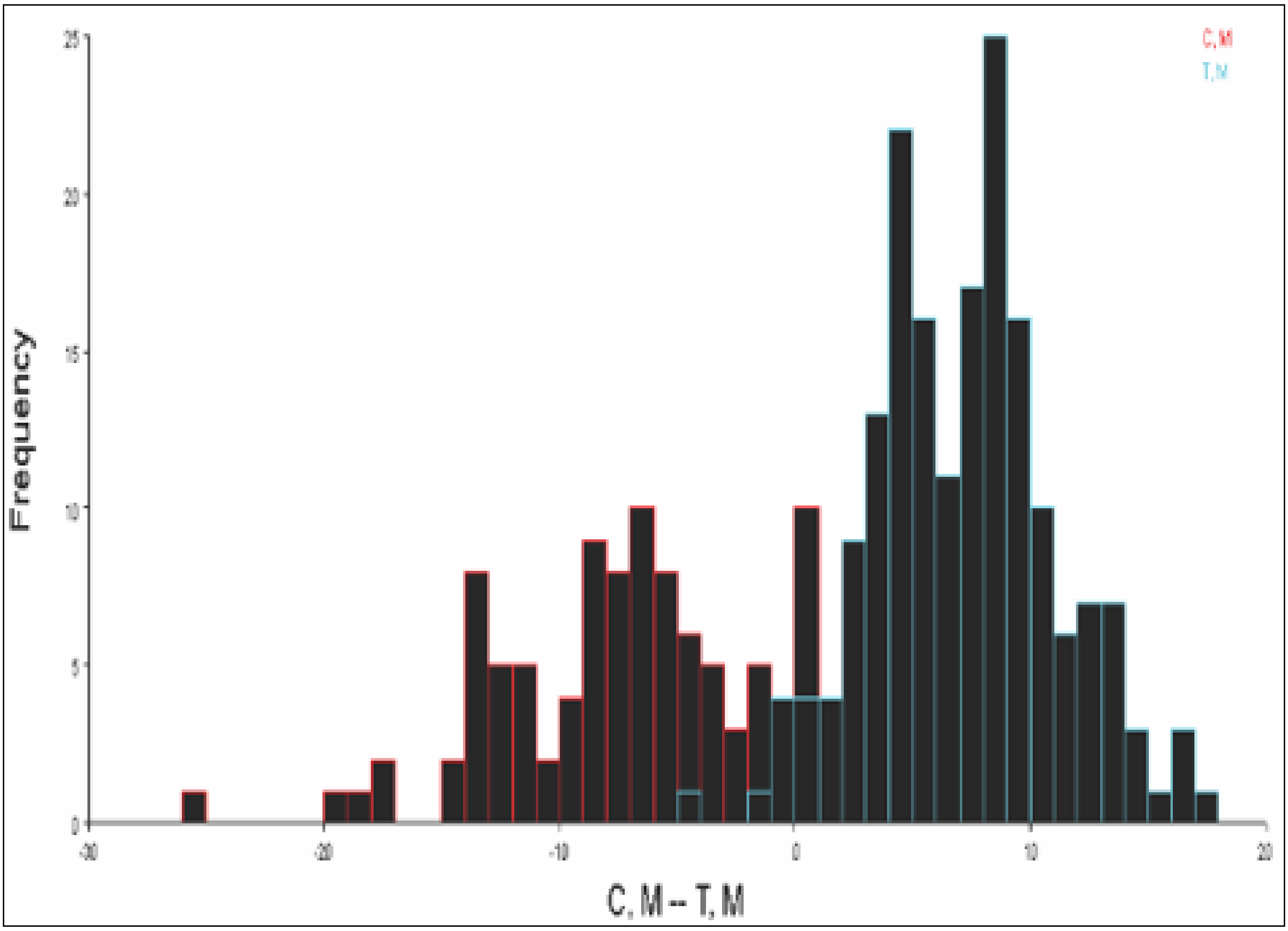


Fig 5: (a)-Discriminant analysis representing female insect hind wings of control and from contaminated area, (b)-Thin plate deformation grid representing female insect hind wings of control and contaminated insects

Uncontaminated and Contaminated Male Hind Wing Size and Shape Variations

In this comparison almost all land marks show deviations except landmark 18 (distal end of anterior anal). All the land marks showed a high degree of deviation in its position. The shape variation was also observed between left and right hind wings of disturbed and undisturbed male, which revealed that they showed asymmetry in forewings. The P value obtained was <.0001, indicating the significant difference (Fig 6).



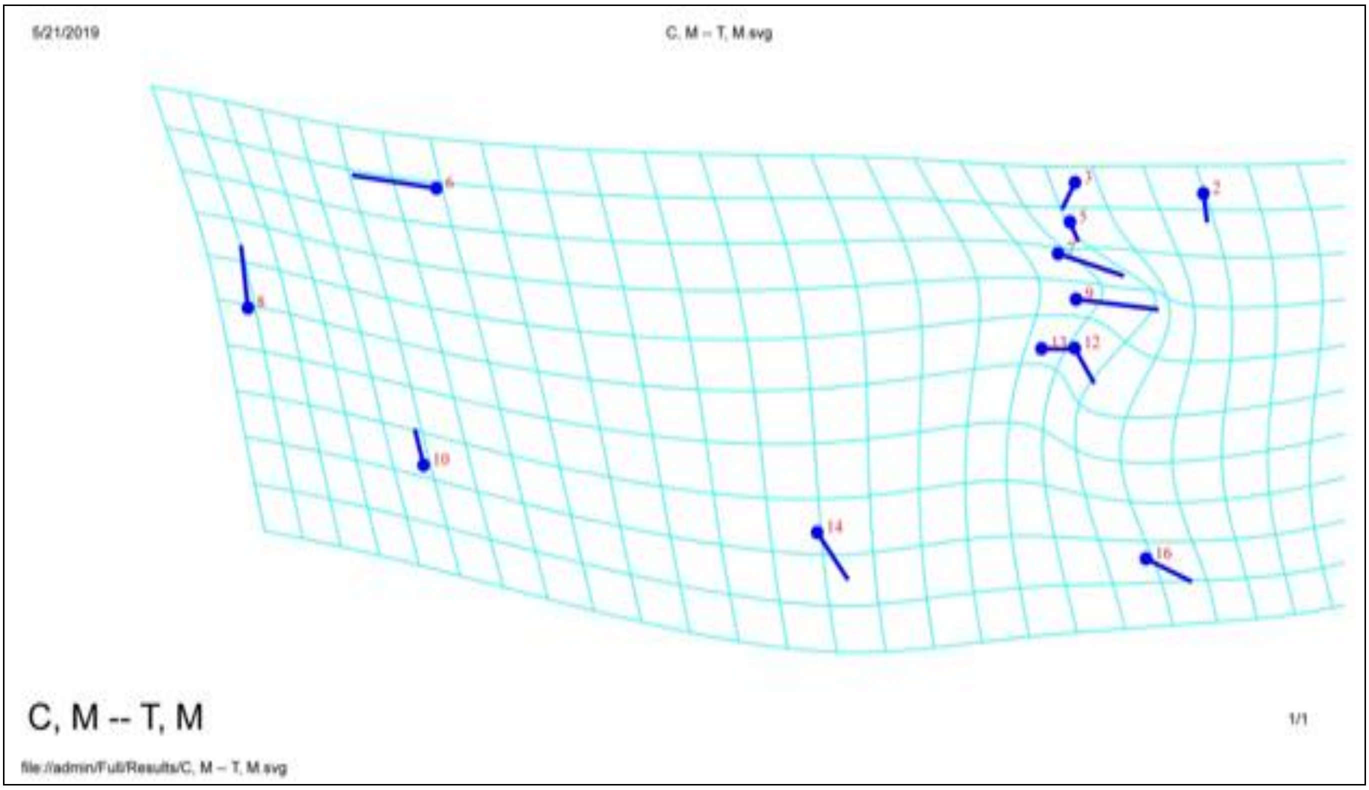
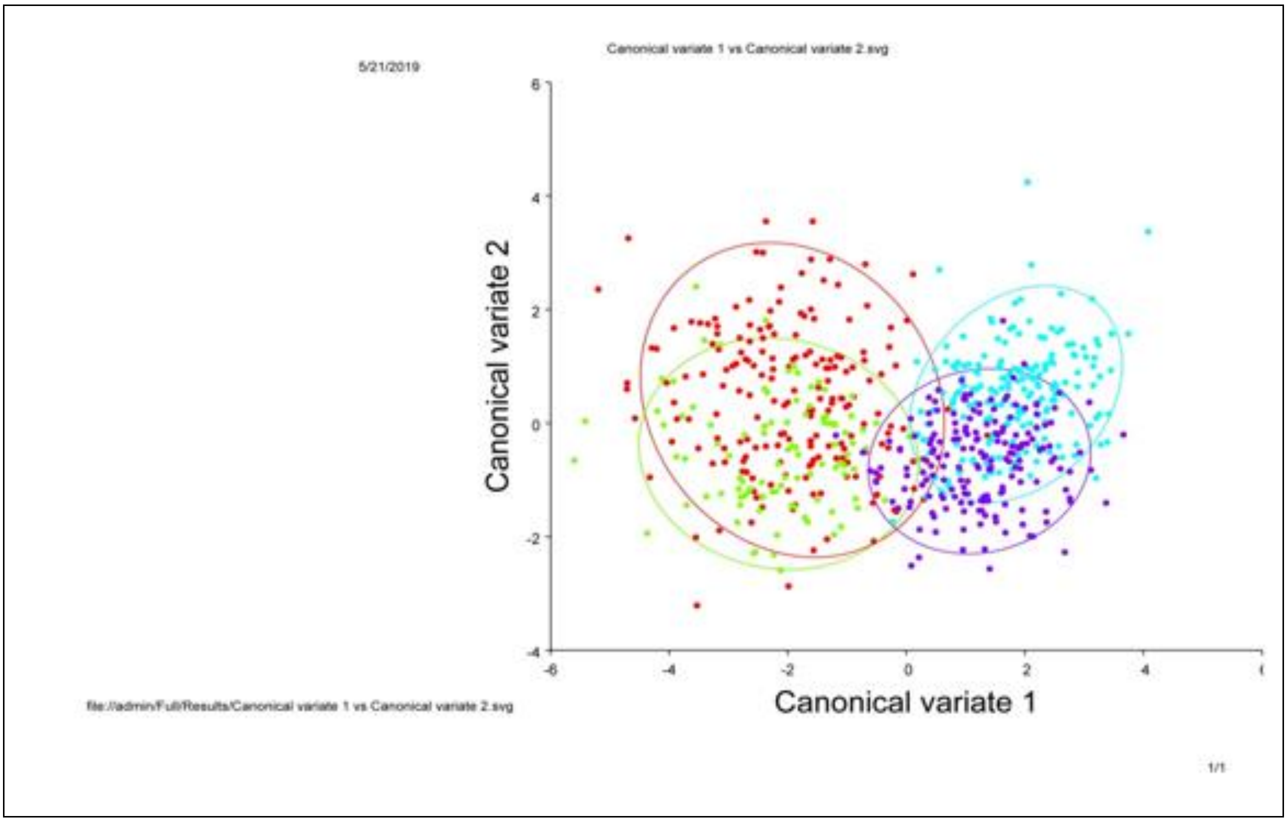


Fig 6: (a)-Discriminant analysis representing male insects, hind wings of control and contaminated area. (b)-Thin plate deformation grid representing male insect hind wings of control and contaminated area.

Canonical Variate Analysis

The canonical variate analysis showed significant change in wing shape. From about 690 observations, 664 are included in the data analysis. Canonical variate analysis contributes 87.047% variation by CV1. The cumulative percentage of variation produced by CV1, CV2, CV3. is 100 (Table 3.5). The thin plate deformation grid shows the variation due to CV1. The canonical variate analysis (CVA) was performed to determine the shape features between multiple groups of the individuals. The scatter plot of CVA represented great shape variations between the control and treated male and female insect hind wings (Fig 7).



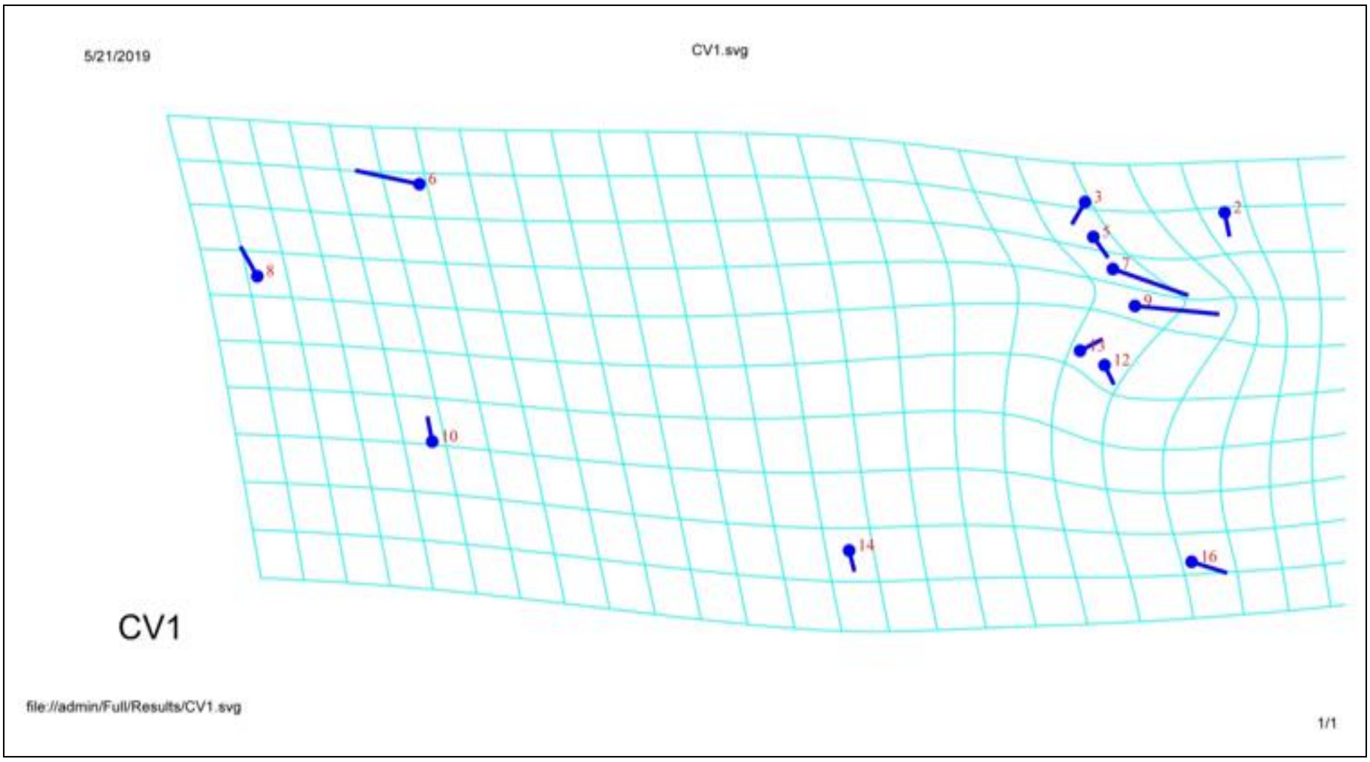


Fig 7: (a) - Canonical variate plot showing the distortion of hind wings of uncontaminated and contaminated insects of both the sexes, based on the shape of left and right sides (b) - canonical variate plot showing the variation by CV1

Discussion

The geometric morphometrics represent a reliable tool in the taxonomic research and also in further study on the evolution of the hind wing shape of coleopteran beetles. Geometric morphometric studies shows that the size and shape of the wing can be used as an indicator of stressful environmental conditions and to evaluate the developmental noise [21].

Recent study on dimorphism by Espra *et al.*[22] in the shape of the wings in *Lucilia sericata* using geometric morphometric methods showed that the variations observed could be genetic or could be mere reflections of the existence of high phenotypic plasticity due to environmental changes during growth and development of the larvae. Using only wing features it is now possible to identify species of various groups of insects with the help of computational morphometric identification systems [23]. From the study it is clear that the male and female hind wing of *Odoiporus longicollis* exhibits wing variation with the p value of 0.0004 and <0.0001 for Procrustes distance and mahalanobis distance of male and female hind wing among the uncontaminated group. The p value for Procrustes distance of male and female hind wing among the contaminated group also is <0.0001. Based on the PCA and CVA study it is very clear that there exists a great variation in hind wing size and shape among and between the uncontaminated and contaminated groups (table 2 & 3). In this study it is identified that most of the landmark variations occurred in distal region than proximal part. Similar studies were also conducted which shows that the apical part of the hind wings of leaf beetles has an important influence on hind wing shape variation[24]. In beetle hind wings are used for flight and it is kept in transversely folded condition under the elytra when not in use. The hind wing as the flight organ, must have a certain size to be aerodynamically functional, which makes them distinctly larger than the thickened fore wings[10] which prove that the areas of the beetle hind wing relevant to transverse folding importantly influence hind wing shape variations. Transverse wing folding is one of the advantages of coleopterans, dermapterans and some species of blattiodea [25]. The CVA results show that the different groups of male and female insects exhibit variation and asymmetry in hind wing, since Mahalanobis and Procrustes distances for each group are significantly different (p<.0001). It also suggests that the hind wing shape is useful for the discrimination of both male and female adult *Odoiporus longicollis* and also with the pesticide affected individuals and the present study on the effect of xenobiotics on the *Odoiporus longicollis* is significant and explains that wing size and shape is influenced by xenobiotics.

Conclusion

The effects of xenobiotic exposure on the wing pattern of the adult banana weevil were investigated using the geometric morphometric method. The wing shape analysis using identified land marks of hind wings reveals cumulative percentage of variation of first three principal components and the results show different groups of male and female insect exhibits variation and asymmetry in hind wing. On the basis of the analysis of the data of the hind wings, it is found that there is a significant variation in the hind wings of both the sexes and also between the insects collected from the pesticides treated and pesticide untreated areas. The hind wing size and shape variation among and between the male and female insects indicate the effect of xenobiotics on the banana pseudo stem weevil and changes wing pattern of *Odoiporus longicollis*.

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