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Research Article

Landmark and outline-based geometric morphometric analysis of wing shape of normal and gamma-irradiated *Ceratitis capitata* (Diptera: Tephritidae)

Fatemeh Farsi, Mehrdad Ahmadi* and Shiva Osouli

Nuclear Agriculture Research School, Nuclear Science and Technology Research Institute, P. O. Box: 31485-498, Karaj, Iran.

Abstract: The geometric-morphometric method has become a vital tool for examining shape and size variations. The current study analyzed the effect of gamma radiation on wing characteristics of sterile and normal Medflies, *C. capitata,* on landmark-and outline-based geometric morphometrics. In landmark-based, there were significant differences in weight matrices as shape variable, but the centroid size as size variable was statistically non-significant between the two populations. PCA of the sterile and normal populations manifested a clear distinction from the results. MANOVA further ascertained significant wing shape differences between sterile and normal populations. In the outline-based approach, PCA of the contours revealed that 10 out of the 80 principal components effectively explained shape attributes. The statistical tests were utilized to introduce the significant influence considered in the sterile insect technique (SIT) because, in the long run, these changes may distort the results of SIT project.

Keywords: geometric morphometric, Outline approach, Landmark approach, sterile insect technique, *C. capitata*

Introduction

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), originally belonged to Africa and then was distributed worldwide (Calla *et al.*, 2014). Medfly inhabits five continents and is known as one of the destructive and economic pests of horticulture (De Meyer *et al.,* 2008). The sterile insect technique (SIT) is used as a component of area-wide integrated pest management (AW-IPM) (Hendrichs *et al.*, 1995; Ahmadi *et al*., 2021). For SIT to be effective, laboratory strains must have sufficient competence in dispersal, survival, and mobility compared to their normal counterparts (Ageep *et al.*, 2014). The wing plays an essential role in the insect's dispersal and mobility. Thus, the wing morphology (size and shape) would be undeniable for optimal flight performance. Among the various roles the wing plays for Tephritidae, their implication in cohort behavior has a special place (Souza *et al.*, 2015). Indeed, the success of mating in males depends on the size of the body and wing (Partidge and Farquhar, 1983; Siomava *et al.*, 2016). The courtship behavior in *C. capitata* involves two models of wing movement, including continuous wing vibration and

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intermittent wing buzzing (Briceno and Eberhard, 2000). Partridge *et al.* (1987) stated that larger males produced more courtship songs and sang more loudly in *Drosophila melanogaster*. In *C. capitata*, males produce a drop of pheromone with a calling song by vibrating their wings in a continuous manner at a fundamental of about 350 Hz; through simultaneous vibration of the wings and the curvature of the abdomen, they direct the pheromone plume to the female. Next, the emission of the wing proceeds at a frequency of 165 Hz. Visual displays of the males are also done with two presentations: the head rapidly rocking on two sides and tapping the female with their aristae (Souza *et al.*, 2015).

Sterilization can target the life parameters of an insect. Calkins and Parker (2005) claimed that the quality of irradiated insects diminished by increasing the radiation dose during sterilization. This quality was considered in the flight ability, longevity, and starting activity. Applying a minimum dose of sterilization is a strategy used to reduce the rate of somatic cell damage in *C. capitata* (Calkins and Parker, 2005). Higher radiation reduces the flying ability of sterile flies, which mitigates their release effect (Guerfali *et al.*, 2011). The radiation damages both sex and somatic cells, causing a disability of sterile flies in producing progeny. Through damage to sex cells, Sterilization occurs with impairing spermatogonia, which interferes with the formation of normal spermatids and sperm (Kruger *et al.*, 2021). Radiation-affected somatic cells show abnormalities, reduction in longevity and flight ability, or even death of the irradiated insect (Kruger *et al.*, 2021). Accordingly, it is very important to examine the radiation dose to reach the appropriate level of sterilization and avoid hurting the ability of sterilized insects, including flight ability and longevity.

Although Mass production of sterile insects can lead to various deformations not detected in the quality control, it would be a major factor that can determine successful copulation with normal females (Souza *et al.*, 2015; Gilchrist and Crisafulli, 2006). Souza *et al.* (2015) illustrated the temporal and spectral parameters between normal and sterile males have different patterns in wing beating, which affect sexual success.

Geometric-morphometric method (GMM) collects an array of extremely adaptable mathematical descriptors of shapes shifting homologous landmarks, surfaces, or outlines into quantitative traits that can be analyzed in the multivariate statistical frame (Wallace *et al.*, 2019). The use of GMM to investigate the effect of radiation on the organs of an organism has been limited (Gilchrist and Crisafulli, 2006; Guefali *et al.*, 2011; Souza *et al.*, 2015). Many studies revealed the use of geometric morphometric-based landmarks, while the number of studies applying the outline-based approach in insects is fewer (Chaiphongpachara and Laojun, 2019; Phanitchat *et al.*, 2019).

According to Cayol (1999), quality control tests, which are mainly based on the evaluation of biological parameters, are insufficient to determine the exact competition of sterile males. Herein, we carried out modern morphometrics, both landmark and outline-based approaches for the shape and size variations of the wing between the sterile and normal males of *C. capitata*.

Materials and Methods

C. capitata consisted of normal males (Group 1) and irradiated sterile males (Group 2) without successful copulation. Both groups originated from the infested Tangerine area (*Citrus tangerine*) in northern Iran, Mazandaran province (36.2262° N, 52.5319° E). Infested fruit samples were collected on first of July 2021. Two Groups used in the experiments were reared as larvae on a diet formulated by Ahmadi *et al.* (2021) as follows: 150 ml of distilled water, 70 g of wheat bran, 17.5 g of brewer's yeast, 35.5 g of sugar, 2.5 g of citric acid, and 2.5 g of benzoate, and an adult diet of protein hydrolysate: sucrose (1:4 w: w) at the Nuclear Science and Technology Research Institute in Karaj, Iran. The condition in the insectary was controlled at 27 °C (± 1 °C) and

70% (± 5%) humidity with a 16L: 8D photoperiod. Further, 3 to 5-day-old pupae (group 2) were irradiated with a ^{60}Co gamma source (PX-30) at a dose of 90 Gy (dose rate of 0.4 Gy/s) (Ahmadi *et al*., 2021).

To avoid any problems related to asymmetry (differences between the left and right-wing) (Fink, 1990), exclusively front wings of the right side of males were detached and used for slide preparation (two treatments × 30 male individuals = 60). High-resolution digital images of wings were captured using a digital camera on a stereo microscope and saved in bitmap format using the computer program DINOCAPTURE V3.2.0.5. To focus more on the details of the wing veins, all images were converted to grayscale using Paint.net software. The TpsUtil, version 1.28 (Rohlf, 2004), which derives the x-y coordinates for each image, was used to randomize the images, and subsequently, TpsDig2, version 2.16 (Rohlf, 2010a) was employed to identify ten anatomically homologous landmarks on the venation of the forewing (Fig. 1; Table 1). The utilized landmarks were recommendations from geometric landmark works in other literature (Pieterse *et al.,* 2017; Lemic *et al.,* 2020; Reis *et al.,* 2021). We examined shape and size variations separately for each wing in both groups. The size of the wings (measurement unit: mm) was computed as the square root of the sum of squared distances of every landmark to their average location, centroid size (CS) (Bookstein, 1997). The use of CS ensures that size is uncorrelated with the shape variations for slight, random, and spherical variations around the landmarks (Bookstein, 1997; Zelditch *et al.,* 2004; Kimmerle *et al.,* 2008). The differences in centroid size were evaluated using a student's t-test. The generalized Procrustes analysis (GPA) was also handled, which makes the sum of squared distances least between corresponding landmarks to extract shape information by eliminating the extraneous information of size, position, and direction (Zelditch *et al.*, 2012). To explain the main trends in shape variations, we performed a principal component analysis (PCA) of the uniform components plus partial warps variables, which were acquired from thin-plate spline analysis. To compare wing shape between groups, A multivariate analysis of variance (MANOVA) was carried out on the landmark data. Subsequently, TpsReg version 1.38 (Rohlf, 2011) was applied to assess the relationship between partial warps and centroid size to determine size-dependent variations. The tpsRelw version 1.49 (Rohlf, 2010b) was used to estimate relative warps (RW), and a scatterplot of the first two warps (including RW1 and RW2) summarized the results.

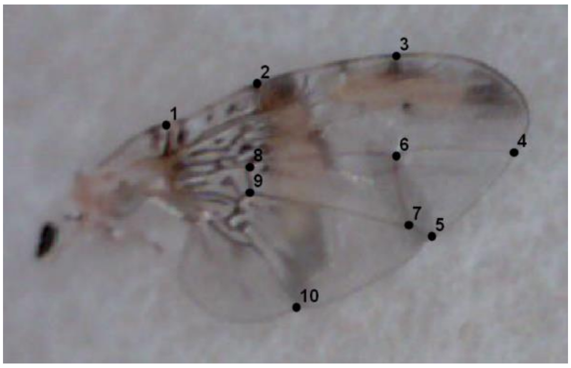

Figure 1 Right-hand wing of *Ceratitis capitata*. Numbers represent the position of landmarks (see Table 1 for description).

Table 1 Descriptions of anatomical landmarks of the right male wing of *Ceratitis capitata.*

Anatomical landmarks	Descriptions
1	The intersection between the humeral and costal veins
2	The intersection between R_1 and costal veins
3	The intersection between R_{2+3} and costal veins
4	The intersection between R_{4+5} and costal veins
5	The intersection between M and apical marginal veins
6	The intersection between r-m and M veins
7	The intersection between dm- cu and CuA_1 veins
8	The intersection between CuA_1 and midcubital distal veins
9	The intersection between r-m and R_{4+5} veins
10	The intersection between the anterior cubital CuA_2 and marginal posterior veins

This study also analyzed the external contour of the front wing using outline methods. In the outline approach, the software package used for shape analysis was SHAPE published by Iwata and Ukai (2002), which contains four programs: ChainCoder, Chc2Nef, PrinCom, and PrinPrint. Initially, converting the images to binary photos in black and white allowed the collection of the contour's chain code using Chc2Nef and calculating the normalized Elliptic Fourier descriptors (EFDs). Afterward, PrinComp performed the PCA of the normalized EFDs. The analysis undertaken was based on the variance-covariance matrix of the coefficient. PrinPrint was the last step that would provide the reconstruction.

Results

The obtained results revealed that the landmark and outline of GMM on wings could distinguish shape changes in normal and sterile populations. Landmarks coordinated for the configuration of *C. capitata* have shown that the accuracy of choosing landmarks was acceptable. All landmarks were located in the target position, and more displacement was seen in the position of a few landmarks (Fig. 2).

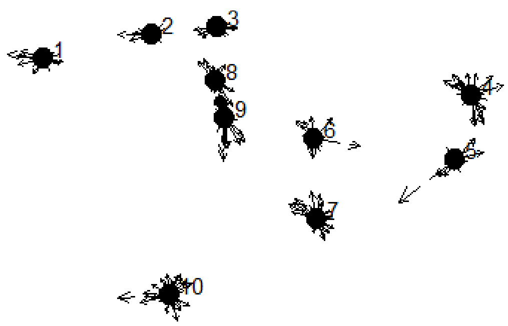

Figure 2 The rate of displacement and changes in the position of landmarks concerning the average shape of the *Ceratitis capitata* studied populations.

The landmark movement from a consensus configuration through deformation grids is evidence of variation (Fig. 3). The analysis demonstrated that 16 RWs accounted for the wing shape variation. RW1 and RW2 explained 49.66% of the variation (RW1 = 31.54%, and RW2 = 18.12%) in the right forewings. The first 10 RWs together accounted for more than 95% of the variation. Although the variation expressed by the ordination plot is scattered to more than two relative warps, the plot of the relative warps 1-2 for the right forewing shows a separation of two medfly populations. Shape variations were considerable via landmarks 5, 6, and 8 (Fig. 3).

Centroid size was used in the present research to measure overall wing size differences between *C. capitata* populations. The mean centroid size did not differ significantly between the normal-type and irradiated medfly populations ($F = 0.776$, $P > 0.382$). Multivariate analysis of variance (MANOVA) indicated that wing morphometric differences between sterile and normal populations of *C. capitata* were significant (Wilks′ = 0.457, $F = 2.071$, $P < 0.05$). This suggests that the difference in the overall shape of the wing between normal and sterile populations of *C. capitata* is definite.

The landmarks and outline-based analysis results showed variations in the shape of the right side of the front wing. In landmark-based, the geometrical attributes of the wing shape were visible using the principal component analysis; it was easily shown that the accumulated variations of shape in the first two principal components reached 42.7% (PC1 = 27.5%; PC2 = 15.2%) (Fig. 4; Table 2). The other components, PC_3 to PC_{16}, explained 57.3% of the total variance. Accordingly, these components have contributed less to explaining the variations across the populations.

In outline-based approach, PCA of the contours showed that 10 of 80 principal components took effect affected to explain shape characteristics. These first 10 PCs described 91.77% of the total variance, with PC1 and PC2 reporting 42.5% and 19.6%, respectively. T-test revealed significant differences between morphological characteristics. Two out of the 10 effective principal components were significantly different between groups (PC1: $F (3, 18) = 23.42$, $p < 005$; PC2: $F (3, 18) = 3.29$, $p < 005$).

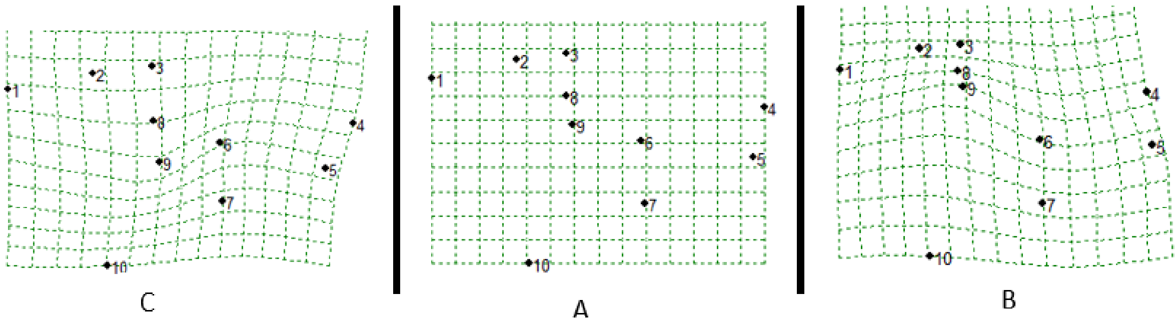

Figure 3 Reference shapes of front wing (A), relative variations in the positive and negative directions of *Ceratitis capitata* population, respectively (B, C).

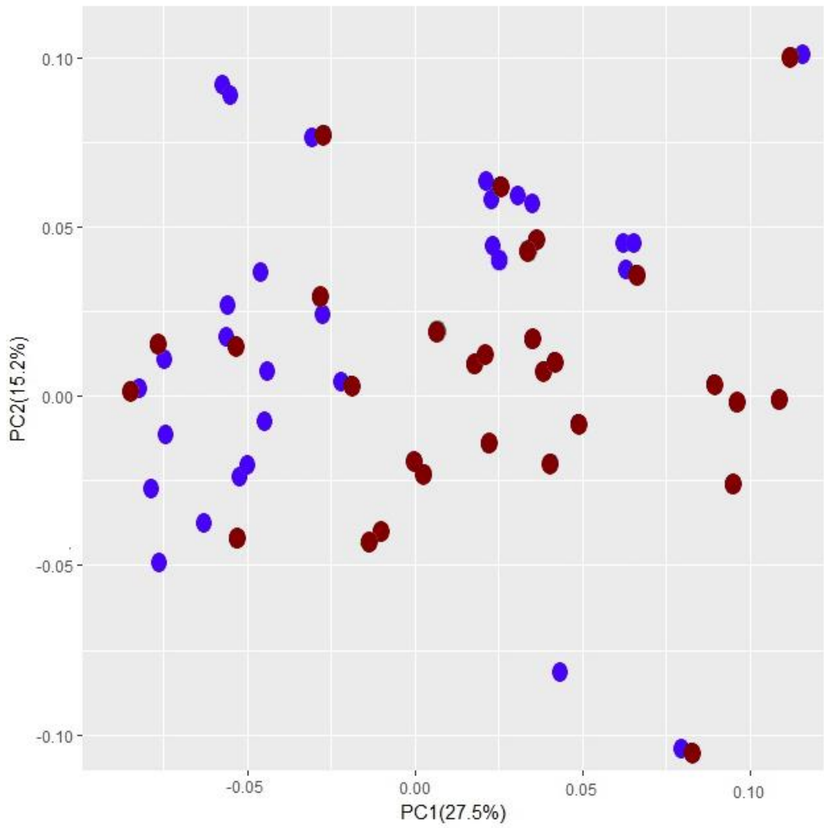

Figure 4 Distribution of *Ceratitis capitata* populations relative to PC1 and PC2 axes. The red circles are the sterile population, and the blue circles are the normal population.

Discussion

The present study found (i) an overall wing size similarity between sterile and normal populations of *C. capitata*, (ii) a significant effect of radiation factors on wing shape traits based on the landmark approach of GMM, (iii) variations in the shape of the two populations' wing based on outline-based geometric morphometric analysis.

Van Cann *et al*. (2015) stated that landmarks related to radial, medial and anal veins are the key anatomical characteristics in Tephritids. In our study, shape variation was noticeable via landmarks 5, 6, and 8 of the veins. Male Medfly has shorter and wider wings than female (Churchill- Stanland *et al*., 1986) that interact with mating and acoustic signal production. Souza *et al*. (2015) studied variation in the wing of the sterile and wild males of *C. capitata* and found that wing shape differed between the two groups. These data are corroborated with those obtained in our study for medfly. Gilchrist and Crisafulli (2006) revealed that wing shape differed between two groups of *Bactrocera tryoni* (Froggatt) (Dip.: Tephritidae). According to these authors' findings, the wing of sterile males varied significantly in width and length compared to the wild group from regions with high altitudes. Morphological changes of the wings were associated with successful mating.

Table 2 Cumulative variance of each principal component variations in the *Ceratitis capitata* forewing.

Component	Standard deviation	Proportion of variance	Cumulative proportion (%)
1	2.09	27.5	27.5
2	1.56	15.2	42.7
3	1.24	9.7	52.5
4	1.22	9.3	61.8
5	1.14	8.2	70.0
6	0.99	6.1	76.2
7	0.87	4.8	81.0
8	0.86	4.7	85.8
9	0.81	4.1	89.9
10	0.53	2.5	92.4
11	0.59	2.2	94.7
12	0.55	1.9	96.6
13	0.48	0.14	98.0
14	0.42	0.13	99.2
15	0.26	0.04	99.6
16	0.23	0.03	100

Wing morphology (size and shape) depends on genetics and other factors, including environment, selective pressures, and random events at the molecular level (Gomez *et al.*, 2014). Environmental changes experienced by immature stages during their development in breeding sites can explain wing size variations (Jirakanjanakit *et al.*, 2007; Paaijmans *et al.*, 2009; Araujo *et al.*, 2012). This also applies to the changes observed in the size of distinct body parts, for example, wings, across individuals of similar species (Reis *et al.*, 2021). Previous investigations have reported that wing size is affected by environmental conditions (Gomez *et al.*, 2014; Lorenz *et al.*, 2017). In this study, radiation, as an environmental factor, only affected the wing shape but not their sizes. Pieterse *et al*. (2017) used GMM to compare the wing attributes of two tephritid species (*Bactrocera dorsalis* (Hendel) and *Ceratitis capitata*) on various hosts. They stated that centroid size, unlike the shape, did not change significantly. Size variations, unlike shape, could be a product of well-coordinated interaction during development, and as such, it has been somehow conservative (Reis *et al.*, 2021). Different wing shapes reflect distinctions in flight range and flight performance that can influence the control of pest species (Wootton, 1981; Pieterse *et al*., 2017). Pieterse *et al.* (2017), referring to a previous study (Wootton, 1981), stated that shape variations of *C. capitata* wing could change its kinetics, which might affect the strength of the wing. Wing shape changes, as evidenced by wing strength and beating patterns, are likely to affect insect call and mating behavior. Souza *et al*. (2015) showed that the morphological character of the wings of *C. capitata* is probably associated with the production of the acoustic signal.

The current investigation was the first geometric-morphometric study based on landmark and outline approaches between sterile and normal medfly wings. Despite significant investments in recent decades and the accumulation of knowledge related to controlling fruit flies by SIT, many aspects, especially those associated with the changes in its morphological traits, remain unknown. Most studies have typically emphasized three main issues: biological parameters, longevity, and flight ability. There is some evidence that medfly traits change due to radiation (Haiba and Mahmoud, 2009; Souza *et al.*, 2015). Variations in the size and shape of the wing may be related to flight requirements such as flight height and speed, all of which affect mating behavior, which is an essential parameter in the success of SIT. Souza *et al*. (2015) stated that any deviation in the

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expression of wild sexual behavior could reduce the effectiveness of SIT by reducing medfly male's reproductive competitiveness.

Wing morphology can affect the aerodynamic ability and flight efficiency of insects (Esterhuizen, 2013). Although the effect of size on mating ability in tephritid species is already proven, the effect of shape changes has not been evaluated much. For example, in the laboratory, *Anastrepha suspense* (Loew) (Dip. Tephritidae) females prefer more prominent and larger-sized males (Burk and Webb, 1983). When a male of this species has a larger wing surface, it produces a louder sound which is very attractive to the female (Churchill- -Stanland *et al.*, 1986).

Conclusion

The wing of males in *C. capitata* is shorter and wider than the females' (Lemic *et al.*, 2020). The male establishes mating territories to call females. Thus, the morphology of the wing is more important in mating and continuation of the generation than its role for movement and displacement. Any changes in the morphological parameters of the wing can affect the calling song, mating, and next-generation reproduction. Our results indicated the influence of the radiation on wing shape changes. Considering the growing trend of using SIT in *C. capitata* control programs, we suggest gathering accurate information about morphological characteristics of mass-reared flies because long-term morphological changes can alter the results of SIT projects

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Conflicts of interest
The authors declare no conflict of interest.

Availability of data and material
The authors declare these data are not in other papers.

Author contribution the authors declare to contribute to this article.

Ethics approval
All authors agree to have participated in this article.

Consent to participate
The authors declare consent for the publication of this article.

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تجزیه‌وتحلیل شکل و اندازه بال مگس میوه *Ceratitis capitata* (Diptera: Tephritidae) طبیعی و تحت تابش گاما براساس روش‌های ریخت‌سنجی هندسی و برون‌خطی

فاطمه فارسی، مهرداد احمدی* و شیوا اصولی

گروه گیاه‌پزشکی، پژوهشکده کشاورزی هسته‌ای، پژوهشگاه علوم و فنون هسته‌ای، کرج، ایران.
پست الکترونیکی نویسنده مسئول مکاتبه: mdaahmadi@aeoi.org.ir
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چکیده: روش ریخت‌سنجی به ابزاری قدرتمند برای ارزیابی تغییرات شکل و اندازه تبدیل شده است. مطالعه حاضر، اثر تابش گاما بر تغییرات ریخت‌سنجی مگس میوه *C. capitata* براساس هر دو روش هندسی و برون‌خطی را در جمعیت‌های طبیعی و پرتودیده تجزیه‌وتحلیل نموده است. در روش هندسی، تفاوت معنی‌داری در ماتریکس وزنی به‌عنوان متغیر شکل وجود داشت درحالی‌که سنتروئید سایز به‌عنوان متغیر اندازه ازنظر آماری بین دو جمعیت، معنی‌دار نبود. تجزیه به مؤلفه‌های اصلی (PCA) در جمعیت‌های طبیعی و پرتودیده تمایز واضحی را نشان داد. MANOVA نیز تفاوت معنی‌داری را در شکل بال جمعیت‌های طبیعی و پرتودیده مشخص نمود. در رویکرد مبتنی بر روش برون‌خطی، PCA کانترها نشان داد که ۱۰ مؤلفه از ۸۰ مؤلفه در تبیین ویژگی‌های شکل مؤثر هستند. این آزمون‌های آماری برای معرفی تأثیرات قابل‌توجهی که بر SIT دارند، مورد استفاده قرار گرفت. زیرا این تغییرات در درازمدت ممکن است نتایج پروژه‌های SIT را مخدوش کنند.

واژگان کلیدی: ریخت‌سنجی هندسی، رویکرد برون‌خطی، لندمارک، SIT، *C. capitata*