Chapter

Threat to *Citrus* in a Global Pollinator Decline Scenario: Current Understanding of Its Pollination Requirements and Future Directions

Subhankar Gurung and Arun Chettri

Abstract

Pollinators are vital for world biodiversity and their contribution to agricultural productivity is immense. Pollinators are globally declining with reports such as colony collapse being documented. *Citrus* exhibits a varying degree of pollination requirements due to its vast cultivars being developed all the time. The article intends to understand the breeding system of a few commercially important *Citrus* groups and discern its dependency on pollination services. The threat related to pollinator decline to the *Citrus* industry is measured not only by its reliance on pollinators but also the requirement of the consumers and manufacturers who mostly seek seedless varieties. Therefore, the threat can be tackled by developing high-quality seedless varieties where pollination requirement is absent. Although the importance of pollinators on several self-incompatible varieties cannot be negated, the impact of pollinator decline on its production will entirely depend upon the demand of the market.

Keywords: breeding system, pollinators, pollination, *Citrus*, fruit set

1. Introduction

Pollinators are insects or vectors that transfer the pollen from the male part of the flower, i.e., anther to the female part of the flower i.e., stigma enabling fertilization and seed production [1, 2]. Pollination is an ecosystem service that enhances crop production and helps in sustaining human life [1, 3]. Furthermore, many wild plants that provide calories and micronutrients to human diets require pollination as well [4]. Approximately, 90% of wild flowering plant species worldwide rely on insect vectors for pollen transmission. These plants are essential for ecosystems to function properly because they supply food, habitat, and other resources to a variety of different species [5]. One-third of the agricultural plants, including the cultivars, are dependent on animal pollinators for their reproduction and increased fruit set [6]. Bees play a pivotal role among pollinators by visiting more than 9% of the leading global crop plants [3]. Unfortunately, agricultural intensification has led to a loss of habitat of many insect pollinators and monoculture plantings have threatened insect biodiversity [7, 8]. A palpable concern for the global decline in pollinators can be sensed [9] which may affect fruit set or seed set [10] influencing its yield [11, 12]. The decline in pollinators would also result in a parallel decline in its associated plant species [9]. Insect pollination is therefore also known as an endangered ecosystem service [13–16]. The pollinator crisis threatens global and local food security and can destabilize human life [17]. Therefore, studies in crop pollination are upfront because of the perceived danger that it invites [16–18]. However, a detailed preview of pollination studies is incomplete or redundant [19]. Therefore, understanding the pollination needs of different cultivated plants is a prerequisite to understanding their dependency on animal pollinators [19].

In this regard, the pollination requirement of *Citrus* has been arising much curiosity. Domestication of *Citrus* sp. along with grafting has resulted in variation in the existing species in comparison to its wild progenitors [20, 21]. As such, the present cultivars exhibit tremendous variation in pollination requirements. *Citrus* was considered as a crop with little or no requirement for insect pollination [22]. Webber and Batchelor [23] emphasized, however, that no variety is ever static, and the possibility that its pollination requirements have changed cannot be ruled out. Further research over the period has proven Webber and Batchelor [23] to be correct. However, it has been found the pollination requirements of *Citrus* have been confusing and misleading. It has been reported that *Citrus* have complete or perfect flowers and generally pollinate themselves to produce fruit [24]. *Citrus* has been reported to exhibit varying breeding systems within species and cultivars of the same species [22, 25]. Pollinator reliance and breeding systems differ between and within *Citrus* species, and even between cultivars of the same species [25].

Several *Citrus* hybrid cultivars also exhibit sexual incompatibility which affects their yield [26]. Further, *Citrus* exhibit parthenocarpy and is an important trait that results in a seedless variety [27, 28]. Therefore, it would not be wrong to say that the pollination requirements for *Citrus* are quite diverse [22, 29]. The reason for such a varying breeding system can be attributed to its numerous varieties and the fact that more are being developed all the time. Moreover, each variety has its characteristics that determine the pollination mechanism in them [29]. For instance, some studies state that cross-pollination is not necessary for fruit sets [25, 30], whereas others showed that insect pollinators increased fruit production [31]. Most seeds are produced by asexual means in some cultivars. However, the benefits drawn from insect variation cannot be negated [22].

Given the complex breeding system of *Citrus*, it is difficult to predict the magnitude of the threat the dwindling pollinator number poses to the productivity of *Citrus*. As a step towards understanding the repercussion, we review the pollination requirements of some of the commercially important *Citrus* cultivars and highlight the danger that the global pollinator decline poses on such *Citrus* varieties.

2. Pollinators and its global decline

Honeybee (*Apis mellifera*) is the primary pollinating agent of *Citrus*. They have shown to be effective pollinators of an array of cultivated crops, oilseeds, forage crops, fiber crops, and cereal crops [32]. Evidence suggests that 59% of the domestic honeybee colonies were lost between 1947 and 2005 [33] and 25% were lost in Europe between 1985 and 2005 [34]. Considerable fear has been sensed about the future availability of honeybee pollination. Further, it was observed that specialists (diet/habitat) and sedentary bees and hoverflies tended to decline while mobile generalists tended to thrive [9]. Globally, *A. mellifera* is the predominantly managed pollinator to augment agricultural productivity [34] and ranks as the most frequent single species of pollinator for crops worldwide [35]. There has been increased mortality of managed *A. mellifera* colonies in some regions of the world [36, 37], which could spread to its wild population [38]. Considering the importance of *A. mellifera* in crop productivity, threats to its population could prove to be a disaster for crop reproduction and its productivity [1]. Further, reports suggest that the floral visits by *A. mellifera* were double that of all bumblebee species combined [38]. *A. mellifera* has also been observed to compete and displace native pollinators [39, 40] or compromise plant reproductive success [41]. Therefore, it is the competition between native and non-native pollinators along with various other factors such as pesticides, pathogens, parasites, and climate change that have been triggering the decline of pollinators worldwide [38].

3. Inflorescence of *Citrus* and its sexual process

A *Citrus* flower usually comprises both male and female sex organs in the same flower [26]. Each flower has radial symmetry and consists of 5–6 sepals and 5 petals (**Figure 1B**). The inflorescence is a cyme or raceme, rarely of solitary flowers. The flowers are usually bisexual and actinomorphic, hypogynous, rarely epigynous. It consists of 4–5 (2–3) sepals or lobes. The corolla is apopetalous or sympetalous with 4–5 (0, 2–3) imbricate or valvate petals or lobes. The stamens are 8–10–∞ usually diplostemonous in 2 (1–4) whorls.

The stigma and ovary are connected by a white style that forms a knob [42]. The pistil is in the center of each flower and is surrounded by a whorl of stamens which consists of long filaments with anthers at the top (**Figure 1A**). The anthers are longitudinal indehiscence and consist of yellow pollen that gets shed on the sticky

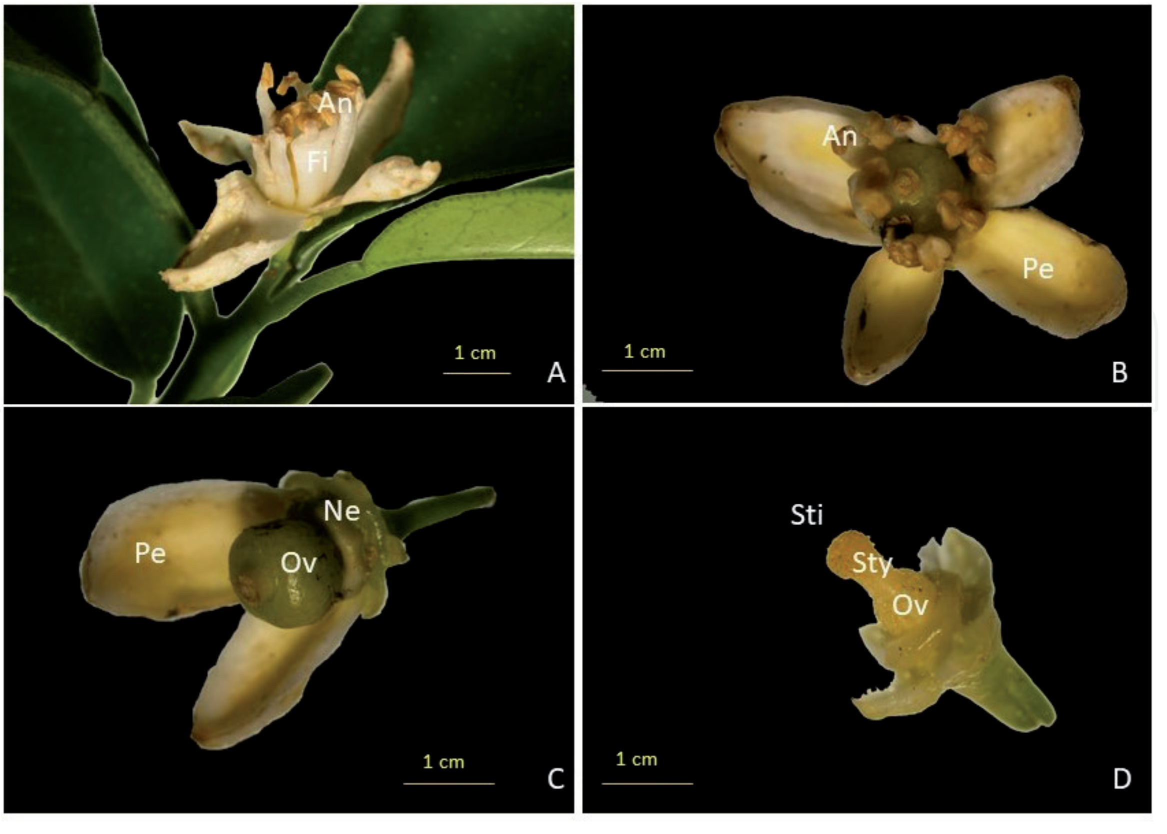

Figure 1.
*Flowers of mandarin (*Citrus reticulata*): (A and B) single flower, (C) fertilized ovary, (D) female sex organ (pistil); An—anther, Fi—filament, Pe—petals, Ov—oary, Ne—nectar, Sti—stigma, Sty—style. ©Subhankar Gurung.*

surface of the stigma [26]. *Citrus* pollen is heavy and sticky [26], and is not adapted for wind pollination [43]. *Citrus* flowers are either self-pollinated, cross-pollinated [43], or can produce fruit by parthenocarpy or nucellar embryony [44]. At the bottom of the ovary, there is a nectary gland. *Citrus* produces nectar copiously [22] and is secreted continuously for at least 48 hours after the flower opening [45].

3.1 Breeding system of mandarin and mandarin hybrid-complex

Several mandarin hybrids of fine quality have been evolved that have become commercially important varieties. For instance, crosses between clementine and tangelo (*Citrus reticulata* × *[Citrus paradisi* × *C. reticulata*]) have produced hybrids such as Robinson, Lee, Osceola, Page, and Nova [46]. King mandarin *Citrus nobilis* proved to be an exceptional parent when crossed with other mandarin types in producing well-flavored hybrids [47]. Tangelo is a cross between tangerine, or mandarin orange (*C. reticulata*) with either grapefruit (*Citrus paradisi*) or pummelo (*Citrus grandis*) [24]. Clementine (*Citrus clementina*) is a hybrid between Mediterranean *Citrus deliciosa* and sweet orange [48]. While the hybrids were being released, issues in its pollination were recognized where the fruit set in Clementine mandarin was fewer in flowers enclosed in self-pollination without bees than those flowers enclosed with bees [49]. Clementine mandarin was identified to be self-incompatible but showed improved fruit characteristics when cross-pollinated with March grapefruit and Balady orange [48]. Besides, it has also been reported that Clementine mandarin develops a seedless fruit if cross-pollination does not occur [50]. Clementine mandarin cultivars (*Citrus clementina*) exhibit facultative parthenocarpy and is pollination independent [50]. However, "Clemenules" exhibits weak parthenocarpic ability than 'Marisol' indicating a difference in the parthenocarpic ability of Clementine cultivars. Further, studies on "Lee", "Page", "Nova" and "Robinson" identified them to be self-incompatible [51]. 'Afourer' mandarin was also found to be self-incompatible and produced relatively high fruit set by facultative and autonomous parthenocarpy [52]. On the contrary, studies demonstrated that "Osceola" was not completely self-incompatible [53]. Further studies showed that "Hyuganatsu" mandarin was self-sterile but cross-fertile [54]. Several experiments demonstrated that "Satsuma" mandarin benefitted 6.3% [55] and 7–11% [56, 57] by bees. They also present obligate parthenocarpy [58], and thus the sexual process is not always required for successful fruit development. There was no significant difference in the final fruit set between parthenocarpic and fertilized Satsuma mandarin [50]. Several mandarin cultivars also benefit tremendously from compatible pollinizers for successful fruit development. For instance: grapefruit pollen is an efficient pollinizer in fertilizing "Clementine" mandarins. Further, field experiments demonstrated that pollens derived from "Lee" enhanced the fruit size of "Page" cultivars [59]. Minneola tangelo increased its yield when cross-pollinated with "Seminole" or "Lake" pollen which indicated that the Minneola fruit set can be enhanced by planting a suitable pollinizer [60].

Some of the accessions of mandarin such as "Kunenbo" are self-incompatible because of incompatible alleles originating from pummelo [61] while mandarins that originated in China and India are self-compatible [62].

3.2 Breeding system of sweet orange

Breeding system of oranges is complex because of the variation in its cultivars. *Citrus sinensis* var. Pera-Rio benefitted from the visitation of bees resulting in heavier, less acidic fruit with fewer seed sets [63]. Further, the fruit production was 35.30% greater in uncovered flowers, fruit weight was higher (180.2 g) than covered ones (168.5 g) [64]. However, bees did not affect the production of *C. sinensis* var. "Valencia" sweet oranges [65, 66]. Sanford [66] also mentions that 'Valencia' does not benefit much from pollination by bees. "Valencia" experienced lower pollen grain germination resulting in a lower seed set [50]. Defective ovule mutants encourage flawed pollen tube guidance [50], and therefore attributed low seed set in "Valencia" sweet orange to defects during female gametogenesis [67]. However, unlike the previous report, fertilization remains an important factor in its improved fruit set [50]. Similarly, ambiguity regarding the pollination of "washington navel" remained when studies demonstrated that cross-pollination might influence fruit set [68]. However, later studies reinstated that cross-pollination does not affect the yield of "washington navels" [69]. Atkins [70] stated that there is a possibility that cross-pollination by bees may cause them to retain more fruit [70]. There was a 31% increase in the fruit set, a 21% increase in fruit weight, 33% more juice, and 36% more seeds from fruits on trees visited by bees [71]. Further "washington navel" is male sterile and is incapable of sexual reproduction when used as a pollen parent [72]. Therefore, the likeliness of it requiring a pollinizer is high for a successful fruit set. In this context, it was observed that "washington navel" produced the most fruits when crossed with "Grusinian" orange [57]. However, the embryo sac of "washington navel" may also degenerate before pollen tubes penetrate through it.

3.3 Breeding system of pummelo

Most of the pummelo varieties are self-incompatible because pollen tubes cannot grow properly because of the genetic barrier to inbreeding [73]. Further, most pummelo trees are self-incompatible and should be inter-planted with other cultivars [74]. Shaddock (*Citrus maxima* L.) or (*Citrus grandis* L.) increased its fruit set %, fruit retention, fruit weight, fruit size when cross-pollinated with Balady orange pollen grains [48]. Further, Aala [74] suggested that bees are important for proper pollination and fruit set irrespective of a cultivar being self-fertile or self-sterile. *Citrus maxima* (burm) Merr cv. *nambangan* is also reported to be self-incompatible and is dependent on insect pollinators for fruit sets [42]. However, Hoang et al. [75] reported that pollen tube growth of *Citrus grandis* cv. "Phuc Trach", "Dien" and "Red Pummelo" were arrested in the style inhibiting fertilization. "Da Xanh" and "Nam Roi" pummelos did not require pollination and produced large-sized seedless fruit by vegetative parthenocarpy [75]. Therefore, it is an established fact that few cultivars of pummelo are highly parthenocarpic [27] and pollination is unnecessary for commercial fruit production.

3.4 Breeding system of grapefruit

The pollination requirements and breeding system of grapefruit are poorly understood [76]. Some studies suggest that cross-pollination is not a prerequisite for fruit set [25, 77], whereas others demonstrate that the transfer of cross-pollen mediated by insects increases fruit production [31]. For example, there are species such as "Red Blush" grapefruit cultivar that is highly parthenocarpic while "Marsh" grapefruit set four times as many fruits in open-pollinated flowers than selfed flowers [78]. "Star Ruby" grapefruit (*C. paradisi* Macf.) consists of non-functional pollen, non-functional ovules and produces more fruits when cross-pollinated [31]. Further, studies suggest that pollen transfer by insects and pollen tube development are important factors for fruit production in grapefruit [76, 79]. Chacoff and Aizen [76] also demonstrated that more cross-pollen tubes reached the ovary than self-pollen, indicating the importance of pollinators in successful fruit development.

3.5 Breeding system of lemons and limes

Lemons (*Citrus aurantiifolia* (Christm.) Swingle) is self-compatible and shows both autogamous and allogamous types of pollination [80]. On the contrary, "Tahiti" lime is strongly parthenocarpic and its dependency on pollination is feeble for increased fruit set [69]. Parthenocarpy in "Tahiti" lime is followed by chromosomal irregularity during meiosis, reflecting the triploid origin of the lines [81]. Likewise, parthenocarpy is high on thornless Rodan lime resulting in a fewer number of seeds [82]. However, the fruit set of *Citrus limettoides* was better in open-pollinated flowers than emasculated or hand-pollinated flowers [83]. Further, the flower-fruit ratio and fruit-seed ratio of lemons declined in the absence of pollinators than in open-pollinated conditions [80]. The yield is further enhanced in lemon with the planting of a suitable pollinizer. For instance, the yield of 'Kagzi Kalan' lemon is supposedly enhanced with the plantation of 10% pummelo along with it [84].

Studies have reported that *Citrus limon* L. was self-incompatible and fruit and seed sets were greater in cross-pollination than self-pollination [85]. Further, it has been observed that pollination by bees is not required for the production of fruits [86]. Further, lemons were reported to be self-compatible that produce fruit by self-pollination without the influence of pollinators [22]. Emasculated unpollinated flowers of *C. limon* produced seedless fruits, indicating its parthenocarpic ability [87]. Although cross-pollination helped *C. limon* produce fruits with germinable seeds, it was self-pollination that produced seedless fruits [87]. However, studies also demonstrated that lemon trees caged without bees produced 42.5% less than open-pollinated trees while trees caged with bees produced 10% less fruit indicating that bees contribute to geitonogamous self-pollination [88].

3.6 Breeding system of citrons

Citron is reported to be receptive to the pollen of the same species [89]. Citrons are presumably self-compatible and hence citrons as maternal parents are not common [89, 90]. Citrons have the lowest heterozygosity which results in their high rate of selfing [91]. It has been observed that the male and female organs of Corsican citrons mature simultaneously before the opening of the petals which indicates its self-fertilization mechanism [92]. As a result, citron propagation by seed in Israeli home gardens seldom results in hybrid seedlings [93]. Further, it has also been reported that no pollination problems have been observed on citron [22].

4. Conclusion and future direction

4.1 Status of pollination study and its relevance in *Citrus*

There is enough evidence to show that the global pollinator population is declining which is harming the agricultural productivity of the crop. *Citrus* is an important cash crop and has a varying degree of pollination requirements. Although, several studies have been conducted to ascertain the pollination requirement of *Citrus,* we found uncertainty in the importance of pollination in *Citrus* as far as market demands are concerned. There are several varieties of *Citrus* [23] that are poorly known from the pollinators and pollination requirement [76]. While McGregor [22] stated that few studies have been conducted in other groups of *Citrus*, we are in a much better position now than we have been in the past to understand the pollination requirements of the plants. Nonetheless, considering that new cultivars are developed every time, the study will always be in its infancy requiring constant monitoring to understand their breeding system. However, we suggest (i) recognizing the market need for *Citrus* varieties of superior eating and processing quality (ii) determining the relevance of pollinators based on prior demand of the market. For instance, consumers' favor seedless fruits because of their appealing appearance and ease of consumption, while producers prefer them due to their ease of processing. Given its relevance, efforts are being done to develop high-quality seedless fruits [58].

4.2 The way forward

In this review, we have explored commercially important *Citrus* species and tried to ascertain their adaptation to pollination requirements. We have also ascertained the breeding system of some of the commercially important *Citrus* varieties around the globe. However, growing evidence suggests that the pollination requirements of *Citrus* may be arguable, the biggest question that arises is the importance of pollination in the *Citrus* industry.

Further, we found a disparity in studies conducted between *Citrus* varieties probably because of the commercial popularity of some varieties over others. We still need studies to be conducted to document important pollinators and understand the breeding system of more varieties. The ever-increasing development of new cultivars poses challenges in understanding the breeding process of such cultivars and a constant effort to study such cultivars is the major challenge that needs to be addressed. It is also evident that there are substantial losses of pollinators in many regions of the globe, therefore segregating *Citrus* varieties and cultivars based on its breeding adaptation, pollination requirements, and most importantly based on the demand of the market would greatly determine the effect of the loss of pollinators in the productivity of *Citrus*. However, if the lack of pollinators does affect productivity and disrupts its market supply, targeting on development of seedless varieties can be a good alternative. Also, genetically self-incompatible cultivars that are dependent on pollinators need to be consistently investigated and their pollinators identified. Identifying suitable pollinizers can be another way in enhancing the productivity of *Citrus*. However, such a process also requires pollinators, and therefore, given the importance of pollination, efforts should be made in mitigating pollinator loss to sustainable pollination service. This can be achieved by acknowledging their intrinsic value, considering the core importance of pollinators for the productivity of *Citrus*, focusing on agri-environment schemes, protected area networks, and managing alternative pollinators.

Acknowledgements

The authors are grateful to the Department of Botany and the Central library of Sikkim University for providing access to the journals. The review was prepared without any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare no conflict of interest.

Author details

Subhankar Gurung and Arun Chettri*
Taxonomy and Biodiversity Lab, Department of Botany, Sikkim University, Gangtok, Sikkim, India

*Address all correspondence to: achettri01@cus.ac.in

References

[1] Potts SG, Imperatriz-Fonseca V, Ngo HT, Aizen MA, Biesmeijer JC, Breeze TD, et al. Safeguarding pollinators and their values to human well-being. Nature. 2016;**540**:220-229. DOI: 10.1038/nature20588

[2] Das A, Sau S, Pandit MK, Saha K. A review on: Importance of pollinators in fruit and vegetable production and their collateral jeopardy from agro-chemicals. Journal of Entomology and Zoology Studies. 2018;**6**(4):1586-1591

[3] Klein AM, Vaissiere BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, et al. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society of London B: Biological Sciences. 2007;**274**:303-313

[4] Sundriyal M, Sundriyal RC. Wild edible plants of the Sikkim Himalaya: Nutritive values of selected species. Economic Botany. 2004;**58**:286-299. DOI: 10.1663/0013-0001(2004)058[0286,WEPOTS]2.0.CO;2

[5] IPBES. The Assessment Report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on Pollinators, Pollination and Food Production. Bonn, Germany: Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services; 2016

[6] Greenleaf SS, Kremen C. Wild bees enhance honey bees' pollination of hybrid sunflower. Proceedings of the National Academy of Sciences of the United States of America. 2006; **103**:13890-13895. DOI: 10.1073/pnas.0600929103

[7] Krebs JR, Wilson JD, Bradbury RB, Siriwardena GM. The second silent spring? Nature. 1999;**400**:611-612

[8] Tscharntke T, Kelin AM, Kruess A, Steffan-Dewenter I, Thies C. Landscape perspective on agricultural intensification and biodiversity-ecosystem service management. Ecology Letters. 2005;**8**:857-874

[9] Biesmeijer JC, Roberts SPM, Reemer M, Ohlemüller Edwards M, Peeters T, Schaffers AP, et al. Parallel declines in pollinators and insect-pollinated plans in Britain and the Netherlands. Science. 2006;**313**:351-354

[10] Rathcke B. Competition and facilitation among plants for pollination. In: Real L, editor. Pollination Biology. London, UK: Academic Press; 1983. pp. 305-329

[11] Rodrigo J, Herrero M. Evaluation of pollination as the cause of erratic fruit set in apricot 'Moniqui'. Journal of Horticultural Science. 1996;**71**:801-805

[12] Partap UMA, Partap TEJ, Yonghua, HE. Pollination failure in apple crop and farmers' management strategies in Hengduan Mountains. China: Acta Horticulturae. 2000;**561**:225-230

[13] Corbet SA. Which bees do plants need? In: Matheson A, Buchmann SL, O'Toole C, Westrich P, Williams JH, editors. The Conservation of Bees. London, UK: Academic Press; 1991. pp. 105-114

[14] Williams IH. The dependence of crop production within the European Union on pollination by honeybees. Agricultural Zoological Review. 1994;**6**:229-257

[15] Kevan PG, Philips T. The economics of pollinator declines: Assessing the consequences. Conservation Ecology. 2001;**5**:8

[16] Ghazoul J. Buzziness as usual? Questioning the global pollination crisis. Trends in Ecology and Evolution. 2005; **20**:367-373

[17] van der JP S, Vaage NS. Pollinators and global food security: The need for holistic global stewardship. Food Ethics. 2016;**1**:75-91

[18] Steffan-Dewenter I, Potts SG, Packer L. Pollinator diversity and crop pollination services are at risk. Trends in Ecology and Evolution. 2005;**20**:651-652

[19] Klein AM, Vaissiere BE, Cane J, Steffan-Dewenter I, Cunningham S, Kremen C, et al. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society of London B: Biological Sciences. 2006;**274**(1608):303-313. DOI: 10.1098/rspb.2006.3721

[20] Casas A, Valiente-Banuet A, Rojas-Martinez A, Dávila P. Reproductive biology and the process of domestication of the columnar cactus *Stenocereus stellatus* in central Mexico. American Journal of Botany. 1999; **86**:534-542

[21] Casas A, Otero-Arnaiz A, Perez-Negron E, Valiente-Banuet A. In situ management and domestication of plants in Mesoamerica. Annals of Botany. 2007;**100**:1101-1115

[22] McGregor SE. Insect pollination of cultivated crop plants. In: Agriculture Handbook 496. USA: USDA-ARS; 1976. p. 395

[23] Webber J, Batchelor LD. The Citrus Industry. 2nd ed. Berkeley and Los Angeles: University of California Press; 1943

[24] Halder S, Ghosh S, Khan R, Khan AA, Perween T, Hasan MA. Role of pollination in fruit crops: A Review. The Pharma Innovation Journal. 2019;**8**(5):695-702

[25] Roubik DW. Pollination of Cultivated Plants in the Tropics. Rome: FAO Agricultural Service Bulletin; 1995

[26] Futch SH, Jackson LK. Pollination of Citrus Hybrids. Florida Cooperative Extension Service IFAS. USA: University of Florida; Fact Sheet No. HS-102. 2003. p. 2

[27] Iwamasa M, Oba Y. Seedlessness due to self-incompatibility in Egami-Buntan, a Japanese pummel cultivar. Agricultural Bulletin of Saga University. 1980;**49**:39-45

[28] Li SJ. Self-incompatibility in 'Matou' wentan (*Citrus grandis* (L.) Osb.). HortScience. 1980;**15**:298-300

[29] Sanford RL, Paaby P, Luvall JC, Phillips E. Climate, geomorphology and aquatic ecosystems. In: McDade LA, Bawa KS, Hespenheide HA, Hartshorn GS, editors. La Selva: Ecology and Natural History of a Neotropical Rain Forest. University of Chicago Press, Chicago, Illinois: USA; 2003. pp. 161-182

[30] Krezdorn AH. Citrus Flowering, Fruit Set and Development. Lake Alfred, FL: Citrus Research and Education Center; 1986

[31] Burger DW. Pollination effects on fruit production of 'Star Ruby' grapefruit (*Citrus paradisi* Macf.). Scientia Horticulturae. 1985;**25**:71-76

[32] Rahman A. Apiculture. In: Kumar AT, Pradhan S, editors. Handbook of Agriculture. Indian Council of Agricultural Research: New Delhi; 2006. pp. 509-529

[33] van Engelsdorp D, Hayes J Jr, Underwood RM, Pettis J. A survey of honeybee colony losses in the U.S., Fall 2007 to Spring 2008. PLos One. 2008;**3**:e4071. DOI: 10.1371/journal.pone.0004071

[34] Potts SG, Roberts SPM, Dean R, Marris G, ABrown M, Jones R, et al. Declines of managed honeybees and beekeepers in Europe? Journal of Apicultural Research. 2010;**49**:15-22

[35] Garibaldi LA et al. Wild pollinators enhance fruit set of crops regardless of honeybee abundance. Science. 2013;**339**:1608-1611

[36] Thompson CE, Biesmeijer JC, Allnutt TR, Pietravalle S, Budge GE. Parasite pressures on feral honeybees (*Apis mellifera* sp.). PLoS One. 2014;**9**:e105164. DOI: 10.1371/journal.pone.0105164

[37] Kraus B, Page REJ. Effect of *Varroa jacobsoni* (Mesostigmata: Varroidae) on feral *Apis mellifera* (Hymenoptera: Apidae) in California. Environment Entomology. 1995;**24**:1473-1480

[38] Hung JKL, Kingston JM, Albrecht M, Holway DA, Kon JR. The worldwide importance of honeybees as pollinators in natural habitats. Proceedings of Royal Society B. 2017;**285**:20172140. DOI: 10.1098/rspb.2017.2140

[39] Thomson D. Competitive interactions between the invasive European honeybee and native bumblebees. Ecology. 2004;**85**:458-470. DOI: 10.1890/02-0626

[40] Thomson DM. Local bumblebee decline linked to recovery of honeybees, drought effects on floral resources. Ecology Letters. 2016;**19**:1247-1255. DOI: 10.1111/ele.12659

[41] Gross CL, Mackay D. Honeybees reduce fitness in the pioneer shrub *Melastoma affine* (Melastomaceae). Biological Conservation. 1998;**86**:169-178. DOI: 10.1016/SOOO6-3207(98)00010-X

[42] Cholis MN, Atmowidi TA, Kahono S. The diversity and abundance of visitor insects on pummel (*Citrus maxima* (burm) Merr) cv. Nambangan. Journal of Entomology and Zoology Studies. 2020;**8**(4):344-351

[43] Free JB. Insect Pollination of Crops. New York: Academic Press; 1970

[44] Zhang S, Liang M, Wang N, Xu Q, Xiuxin D, Chai L. Reproduction in woody perennial Citrus: An update on nucellar embryony and self-incompatibility. Plant Reproduction. 2018;**31**:43-57

[45] Hearn GH, Watkins WG, Bishop RK. Orange nectar and pollen in relation to bee activity. Journal of Economic Entomology. 1942;**35**:321-323

[46] Reece PC, Hearn CJ, Gardner FE. Nova tangelo—an early maturing tangerine hybrid. Proceedings of the Florida State Horticultural Society. 1964;**77**:109

[47] Cameron JW, Frost HB. Genetics, breeding and nucellar embryony. In: Reuther W, Webber HJ, Batchelor LD, editors. The Citrus Industry. Revised ed. Vol. II. Berkely: Div. Agric. Sci., University of California; 1968, Chapter 5

[48] Atawia AR, Abd EL-Latif FM, EL-Badawy HE, Abo-Aziz AB, Abou Rayya MSM, Baiea MHM, et al. Effect of various pollination treatments on yield characteristics and fruit quality of shaddock fruits. International Journal of Scientific & Engineering Research. 2016;**7**(5):1-9

[49] Lacarelle A, Miedzyrzecki C. New contributions on the study of clementines, Morocco. In: Experimentation Fruitiere et Maraichere, Rabat, Morocco. Edition Terre Marocaine ed. France; 1937. p. 22

[50] Mesejo C, Martinez-Fuentes A, Reig C, Agusti M. The effective pollination period in 'Clemenules' mandarin, 'Owari' *Satsuma mandarin* and 'Valenia' sweet orange. Plant Science. 2007;**173**(2):223-230

[51] Hearn CJ, Reece PC. Pollination needs of Page, Lee, Nova and Robinson Citrus hybrids. Citrus Indus. 1967;**48**(4):19-23

[52] Gambetta G, Gravina A, Fasiolo C, Fornero C, Galiger S, Inzaurralde C, et al. Self-incompatibility, parthenocarpy and reduction of seed presence in 'Afourer' mandarin. Scientia Horticulturae. 2013;**164**:183-188

[53] Reece PC, Register PD. Influence of pollinators on fruit set of Robinson and Osceola tangerine hybrids. Florida State Horticultural Society Proceedings. 1961;**74**:64-106

[54] Miwa T. On pollination, fertilization phenomena and problems connected with fruit drop in the hyuganatsu mandarin. Bulletin of the Miyazaki University. Natural Science. 1951;**2**:2-67

[55] Zhgenti SK. Pollination of the Japanese persimmon and mandarin orange. In: Krischunas IV, Gubin AF, editors. Pollination of Agricultural Plants. Russia: Moskva, Gos. Izd-vo SelKhoz Lit-ry; 1956. pp. 193-199

[56] Zavrashvili RM. Importance of nectar in flowers of mandarin orange Unshiu. Soobscenija Akademii Nauk Gruzinskoj. 1967;**45**:1, 205-212

[57] Zavrashvili RM. Influence of bees on the yield of citrus trees on the commercial plantations of Georgia. In: 21st International Apiculture Congress Proceedings College Park, Md. USA. 1967. pp. 450-451

[58] Vardi A, Levin I, Carmin N. Induction of seedlessness in citrus: From classical techniques to emerging biotechnological approaches. Journal of the American Society for Horticultural Sciences. 2008;**133**:117-126

[59] Hearn CJ, Reece PC, Fenton R. Self-incompatibility and the effects of different pollen sources upon fruit characteristics of four citrus hybrids. In: 1st International Symposium Citrus Symposium Proceedings. USA: University of California; Vol. 1. 1969. pp. 183-187

[60] Mustard MJ. Progress Report on the Unfruitfulness of the Minneola Tangelo; Ceiba. 1961;**9**(1):45-48

[61] Ngo BX, Wakana A, Park SM, Nada Y, Fukudome I. Pollen tube behaviours in self-incompatible and self-compatible *Citrus* cultivars. Journal of the Faculty of Agriculture, Kyushu University. 2001;**45**:443-457

[62] Yamamoto M, Kubo T, Tominaga S. Self- and cross-incompatibility of various *Citrus* accessions. Journal of the Japanese Society for Horticultural Science. 2006;**75**(5):372-378

[63] Malerbo-Souza DT, Nogueira-Couto RH, Couto LA. Pollination in orange sweet crop (*Citrus sinensis* L. Osbeck, var. Pera-Rio). Brazilian Journal of Veterinary Research and Animal Science. 2003;**40**(4):237-242. DOI: 10.1590/S1413-95962003 00040001

[64] Malerbo-Souza DT, Nogueira-Couto RH, Couto LA. Honeybee attractants and pollination in sweet orange, *Citrus sinensis* (L.) Osbeck, var. Pera-Rio. Journal of Venomous Animals and Toxins including Tropical Diseases. 2004;**10**(2):144-153

[65] Francke R, Jorge A, Mathieu JM. Effects of insect pollinators on the production of valencia oranges: The honey bee and its effects on citrus production. Agronomia (Monterrey). 1969;**122**:7

[66] Sanford MT. RF-AA092. Pollination of Citrus by Honeybees. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences. USA: University of Florida; 1992

[67] Koltunow AM, Soltys K, Nito N, McClure S. Anther, ovule, seed and nucellar embryo development of *Citrus sinensis* cv. Valencia. Canadian Journal of Botany. 1995;**73**:1567-1582

[68] Krezdorn AH. Fruit setting problems in Citrus. American Journal of Horticultural Science Carribean Region Proceedings. 1965;**9**(13):85-92

[69] Krezdorn AH. Pollination requirements of Citrus. In: The Indispensable Pollinators. Vol. 127. USA: Arkansas Agriculture Extension Service Misc. Pub.; 1970. pp. 211-218

[70] Atkins EL. Honeybees and agriculture. California Citrograph. 1963;**49**(2):81-82

[71] Wafa AK, Ibrahim SH. Effect of the honeybee as a pollinating agent on the yield of orange. Elfelaha. Egypt: Cairo University; 1960:AA-448/63

[72] Davenport TL. Citrus flowering. Horticultural Reviews. 1986;**12**:349-408

[73] Busch JW, Schoen DJ. The evolution of self-incompatibility when mates are limiting. Trend in Plant Science. 2008;**13**(3):128-136

[74] Aala FT. Effects of Hand pollination on the production of Siamese pummel. Phillipines Journal of Agriculture. 1953;**18**(1-4):101-113

[75] Hoang TT, Nguyen TH, Le HT, Ngo BX, Wakana A. Self-incompatibility in pummel [*Citrus grandis* (L.) Osbeeck] with focus on Vietnamese cultivars with or without parthenocarpy. Journal of the Faculty of Agriculture, Kyushu University. 2014;**59**:65-70

[76] Chacoff NP, Aizen MA. Pollination requirements of pigmented grapefruit (*Citrus paradisi* Macf.) from Northwestern Argentina. Crop Science. 2007;**47**:1-9

[77] Krezdorn AH. Pollination requirements of Citrus. Citrus Indus. 1972;**53**:5-7, 28

[78] Wright N. Pollination and the seediness of marsh grapefruit. Proceedings of the Agricultural Society of Trinidad and Tobago. 1937;**37**:51-60

[79] Crane E, Walker P. Pollination Directory for World Crops. Bucks, UK: International Bee Research Association; 1984

[80] Karmakar P. Pollination biology of *Citrus aurantiifolia* (Christm.) Swingle: A medicinally important fruit plant. International Journal of Innovative Research and Development. 2013; **2**(7):138-142

[81] Soost RK, Cameron JW. 'Oroblanco' a tripliod pummel-grapefruit hybrid. HortScience. 1980;**15**:667-669

[82] Abrahimy Y, Haji VS. Comparison of Minab Lime, Rodan Lime and Persian Lime Cultivars on Different Citrus Rootsocks in Minab, Hormozgan, Iran. Bandar Abas (Iran): Iran Citrus Research Institute—ICRI; 2004. Available from: http://www.agrisis.org

[83] Motial VS. Fruit-set studies in sweet lime. Indian Academy of Science Proceedings Section B. 1964; **60**(6):371-379

[84] Haldhar SM, Kumar R, Samadia DK, Singh B, Singh H. Role of insect pollinators and pollinizers in arid and semi-arid horticultural crops. Journal of Agriculture and Ecology. 2018;**5**:1-25

[85] Hossain MI, Rabbani MG. Study on the cross compatibility of some lemon genotypes (*Citrus limon* L.). Bangladesh Journal of Agriculture Research. 2011;**36**(2):241-246

[86] Webber HJ. Influence of pollination on set of fruit in citrus. California Citrog. 1930;**15**(7):304:322-323

[87] Zhang SW, Huang GX, Ding F, He XH, Pan JC. Mechanism of seedlessness in a new lemon cultivar

'Xiangshui' [*Citrus limon* (L.) Burm, F.]. Sex Plant Reproduction. 2012; **25**:337-345

[88] Zavrashvili RM. Bees and the citrus crop. Pchelovodstvo. 1964;**84**(8):19

[89] Moore GA. Oranges and lemons: Clues to the taxonomy of *Citrus* from molecular markers. Trends in Genetics. 2001;**50**:670-672

[90] Scora RW. On the history an origin of *Citrus*. Bulletin of the Torey Botanical Club. 1975;**102**:369-375

[91] Barkley NA, Roose ML, Krueger RR, Federici CT. Assessing genetic diversity and population structure in a citrus germplasm collection utilizing simple sequence repeat markers (SSRs). Theoretical and Applied Genetics. 2006;**112**:1519-1531

[92] Luro FN, Venturini G, Costantino J, Paolini J, Ollitrault P, Costa J. Genetic and chemical diversity of citron (*Citrus medica* L.) based on nuclear and cytoplasmic markers and leaf essential oil composition. Phytochemistry. 2012;**77**:186-196

[93] Goldschmidt EE. Citron propagation: Seeds or cuttings? Alon Hanoeta. 2009;**63**:44-46. (Hebrew)