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ORIGINAL RESEARCH PAPER

# Comparative reproduction mechanisms of three species of *Ocimum* L. (Lamiaceae)

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## Abstract
*Ocimum* species have a combination of reproductive system which varies with the locality and cultivar. We have studied here the reproductive mechanisms of five variants of three *Ocimum* species in Nigeria, namely: *Ocimum canum* Sims., *O. basilicum* L., and *O. americanum* L. Flowers from each variant were subjected to open and bagged pollination treatments of hand self-pollination, spontaneous self-pollination and emasculation. All open treatments of the five *Ocimum* variants produced more fruit and seed than the corresponding bagged treatments. The two *O. canum* variants and *O. basilicum* '$b_1$' produced high fruit and seed set in the open and bagged treatments of spontaneous self-pollination. *Ocimum basilicum* '$b_2$' and *O. americanum* produced higher fruit and seed set in the self-pollination open treatment but significantly lower fruit and seed set in the bagged treatment. Fewer fruit and seeds were produced in the emasculated open treatments but none in the emasculated bagged treatments of the five *Ocimum* variants. The floral foragers comprising of bees, wasps and butterflies visited the *Ocimum* species to collect pollen or nectar in the open treatments. The two *O. canum* variants and *O. basilicum* '$b_1$' variant reproduced mainly through autogamy but *O. basilicum* '$b_2$' and *O. americanum* showed mixed reproduction of autogamy and outcrosssing. Insect visitation to the flowers enhanced pollination resulting in higher fruit and seed set in all the *Ocimum* species studied.

## Keywords
*Ocimum*; insects; pollination; variant; fruit set; seed set; autogamy; outcrossing

## Introduction

The genus *Ocimum* L. (basil) belongs to the family Lamiaceae and comprises approximately 30 species of herbs and subshrubs distributed across tropical regions of Asia, Africa, Central and South America, with Africa as the primary center of diversity of the genus [1]. In Nigeria *Ocimum* is represented by four species, namely *Ocimum basilicum* L., *O. canum* Sims., *O. gratissimum* L., and *O. suave* Willd. [2]. The genus *Ocimum* is an important essential oil crop with around 100 tons of essential oil being produced annually throughout the world, about half of this is produced from *O. basilicum* and close relatives [3]. *Ocimum* species are also very important in indigenous systems of medicine [4–8]. In addition to these uses, *Ocimum* is widely cultivated as a potherb for culinary uses [9] and for the fresh herb market for which export of *Ocimum* from Israel alone is worth 4 million US dollars per year. Floral nectar and pollen are offered as a reward for the biotic pollinating agents [10]. The pollen and nectar of members of the genus *Ocimum* are known to support certain potential pollinators of several wild plants species when the latter are not in bloom [11]. Insect and other organisms play major role in boosting agricultural production by significantly increasing the yields of crops, vegetables, fruit and seeds by visiting flowers to aid

pollination processes. Self-incompatible and cross-pollinated crops require pollinating service of efficient pollinators. Self-pollinated crops also benefit from insect pollination which increases yield up to 30%. Lack of pollinators causes decline in fruit and seed production [12]. There are various reports on the pollination system in species of the family Lamiaceae. According to Darrah [13] and Khosla [14] species of Lamiaceae are primarily crossbreeding and have floral structures suitable for pollination by bees. Raju [11] reported that *O. americanum* and *O. basilicum* are predominantly autogamous. A study performed by Almeida et al. [15] revealed that *O. officinalis* L. is predominantly autogamous but also able to reproduce by cross-fertilization. The floral structure of *O. basilicum* is adapted to pollinator-mediated out-crossing [16]. The pollination mechanisms and plant-insect interactions of the Lamiaceae species vary greatly with the locality and cultivar concerned, ideally pollination investigations are necessary in each locality where the species are grown. Also due to the extensive uses and economic importance of the species in the *Ocimum* genus there is need to have better understanding of the reproductive mechanism in this taxon. This study compared the mode of pollination in three *Ocimum* species: (*O. canum* '$c_1$' and *O. canum* '$c_2$'), *O. basilicum* (*O. basilicum* '$b_1$' and *O. basilicum* '$b_2$') and *O. americanum*, type of floral visitors associated with these *Ocimum* species, and the effect of insects on fruit and seed production.

## Material and methods

This study was conducted in the Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria, located at N07°31 146′, E 004° 31 583′, 287 m a.s.l., between April and August 2013. The *Ocimum* species used in this study included two variants of *O. canum* Sims. (*O. canum* '$c_1$' and *O. canum* '$c_2$') two variants of *O. basilicum* L. (*O. basilicum* '$b_1$'and *O. basilicum* '$b_2$') and one variant of *O. americanum* L. The *Ocimum* variants used here were selected from germplasm collections of the genetic unit of the Department of Botany, Obafemi Awolowo University, Ile-Ife. *Ocimum canum* '$c_1$' is a tetraploid with chromosome number of $2n = 52$ and *O. canum* '$c_2$' is diploid with chromosome number of $2n = 24$. *Ocimum basilicum* '$b_1$' is tetraploid with chromosome number of $2n = 52$ and *O. basilicum* '$b_2$' is a hexaploid with chromosome number of $2n = 72$. *Ocimum americanum* is a tetraploid with chromosome number of $2n = 52$. The seeds of each *Ocimum* variants were raised separately on moistened filter paper in 100 × 15 mm Petri-dishes at room temperature in the laboratory. At the four leaves stage, seedlings were transferred into small plastics cups containing topsoil and were kept in the screen house for the seedlings to be established. Three weeks after germination, seedlings were transplanted one plant per bucket into 7-liters plastic bucket filled with topsoil using five replicates per variant in each treatment.

### Flower morphology

For each variant, the anthers color was determined before anther dehiscence and the number of male and female reproductive structures per flower were also determined by observing the flower bloom each day. The length of twenty stamens and carpel were measured separately on each of the *Ocimum* variants and compared. In each of the variants, the time of opening and closing of flowers and anther dehiscence were observed three times a week between 6 a.m. and 7 p.m. over a period of 2 months. The arrangements of the floral parts immediately after anthesis and after pollination were also observed.

### Pollination experiment

At anthesis time, five potted plants of each *Ocimum* variant were transferred into a screen house made of wired net of mesh size 0.5 mm to exclude the activity of external pollinators. Also five potted plants of each *Ocimum* variant were left in the open. On

each of the *Ocimum* variants in the open and screen house (bagged), the following pollination treatments were investigated on 200 flowers per treatment. The treatments included: (***i***) emasculation of flower buds before anthesis and anther dehiscence occurs; (***ii***) hand self-pollination of stigma by touching it with pollen from its anther immediately after anther dehiscence; (***iii***) spontaneous self-pollination in which flower buds were untouched. In each of the treatments flowers were examined every day for fruit sets. After fructification, the number of fruit and seeds produced from each treatment in the open and screen house were recorded for each *Ocimum* variant. Statistical analysis was performed using system analysis software (SAS) version 9.2 (SAS Institute, Cary, NC, USA). Data obtained from floral measurements, fruit and seed sets were subjected to one-way analysis of variance and their means were compared using Duncan's multiple range test (DMRT).

### Insect visitor behavior

Observations on the behavior of different foragers were recorded. The observations were carried out five times a week during the day from 6 a.m. to 6 p.m. The foraging period, the part of the plant visited and the type of reward collected by different visitors were recorded through close observations. The time spent on a foraging visit was also recorded. The visiting insects were captured with hand net and identified using identification keys [17,18]. The type of pollen grains picked up by the floral visitors were assessed through body washings and examined under a light microscope and compared with pollen of the *Ocimum* variants.

## Results

### Flower morphology

In each of the *Ocimum* species the terminal inflorescence consist of flowers arranged in whorls and oriented horizontally to the axis. Each of the three *Ocimum* species has white petal, green sepal four filaments with white anther, purple-pigmented style and white style in *O. basilicum* '$b_1$'. The flower of each of the *Ocimum* species opens by the separation of the upper and lower corolla lips. Immediately the style bends down but the stigma remains closed for some time. Later the stigma forms a bi-forked shape in the five *Ocimum* variants. Anther dehiscence occurred before anthesis in *O. canum* '$c_1$' and in two variants of *O. basilicum*. In *O. canum* '$c_2$' and *O. americanum* anthesis occurred before anther dehiscence. Anthesis occurred from 7 a.m. to 2 a.m. in all the *Ocimum* variants. The style is significantly longer than the filament in the five *Ocimum* variants studied (Tab. 1).

### Pollination experiment

The average number and percentage fruit and seed sets produced for each treatment on each *Ocimum* variant in the open treatments and bagged treatments were recorded (Tab. 2). In the three *Ocimum* species all the open treatments produced higher fruit and seed sets than their corresponding bagged treatments. In the spontaneous self-pollination of all the five *Ocimum* variants the open treatments produced significantly higher number of fruit and seed set than in the bagged treatments except in fruit set of *O. basilicum* '$b_1$' which showed no significant difference (Tab. 2). Fruit and seed sets of spontaneous self-pollinated were low in bagged treatments of *O. basilicum* '$b_2$' and *O. americanum* but significantly higher in the open treatment (Tab. 2). In all three *Ocimum* species fruit and seed sets were low in the emasculated open treatment and no seed production was recorded for the emasculated bagged treatment, revealing the necessity of pollination (Tab. 2).

**Tab. 1** Comparative mean length of stamen and style (mm) in each *Ocimum* variant.

| | *O. canum* '$c_1$' | *O. canum* '$c_2$' | *O. basilicum* '$b_1$' | *O. basilicum* '$b_2$' | *O. americanum* |
|---|---|---|---|---|---|
| Stamen length | 8.00 ±0.10b | 4.20 ±0.10b | 7.70 ±0.40b | 8.20 ±0.20b | 4.80 ±0.30b |
| Style length | 9.90 ±0.10a | 5.20 ±0.10a | 9.90 ±1.70a | 10.8 ±1.00a | 8.20 ±0.20a |

Means in the same column followed by the same letter are not significantly different at 0.05 probability.

**Tab. 2** Result of pollination experiment on each *Ocimum* variant.

| Treatments | | *O. canum* '$c_1$' | *O. canum* '$c_2$' | *O. basilicum* '$b_1$' | *O. basilicum* '$b_2$' | *O. americanum* |
|---|---|---|---|---|---|---|
| Spontaneous self-pollinated, open | Fruit set (%) | 98.90 | 100.00 | 90.00 | 90.00 | 82.76 |
| | Mean ±*SE* | 50.00 ±10.30a | 50.00 ±2.43a | 39.75 ±5.65a | 67.00 ±6.04a | 54.000 ±2.27a |
| Spontaneous self-pollinated, bagged | Fruit set (%) | 81.80 | 85.50 | 72.00 | 31.00 | 13.26 |
| | Mean ±*SE* | 39.25 ±13.16b | 43.50 ±2.66a | 24.000 ±2.38b | 28.25 ±8.27b | 12.500 ±2.59c |
| Hand self-pollinated, open | Fruit set (%) | 91.50 | 88.00 | 98.50 | 96.00 | 82.11 |
| | Mean ±*SE* | 45.25 ±16.77ab | 43.75 ±5.64a | 39.25 ±3.64a | 74.50 ±7.89a | 44.750 ±3.09b |
| Hand self-pollinated, bagged | Fruit set (%) | 76.30 | 64.50 | 99.00 | 90.00 | 89.27 |
| | Mean ±*SE* | 38.75 ±14.64b | 32.00 ±5.93b | 37.250 ±0.85a | 58.00 ±4.26a | 58.250 ±4.27a |
| Emasculated, open | Fruit set (%) | 46.00 | 33.00 | 27.50 | 28.00 | 40.74 |
| | Mean ±*SE* | 22.75 ±3.12c | 18.25 ±0.75c | 8.250 ±2.39c | 16.00 ±1.58c | 19.250 ±0.63c |
| Emasculated, bagged | Fruit set (%) | 00.00 | 00.00 | 00.00 | 00.00 | 00.00 |
| | Mean ±*SE* | 00.00 ±0.00d | 00.00 ±0.00d | 00.00 ±0.00c | 00.00 ±0.00c | 00.00 ±0.00d |
| Spontaneous self-pollinated, open | Seed set (%) | 98.90 | 100.00 | 96.25 | 81.75 | 44.25 |
| | Mean ±*SE* | 200.00 ±41.18a | 200.00 ±9.71a | 156.0 ±21.93a | 245.75 ±60.90a | 115.50 ±11.89b |
| Spontaneous self-pollinated, bagged | Seed set (%) | 81.80 | 79.00 | 68.13 | 18.50 | 8.36 |
| | Mean ±*SE* | 157.00 ±50.23b | 168.50 ±14.65b | 91.75 ±10.78b | 62.50 ±14.38b | 31.50 ±7.24c |
| Hand self-pollinated, open | Seed set (%) | 91.30 | 58.10 | 98.50 | 90.13 | 55.05 |
| | Mean ±*SE* | 181.00 ±67.05ab | (113.50 ±19.40c | (157.00 ±14.5a | (279.75 ±34.35a | (120.00 ±4.89b |
| Hand self-pollinated, bagged | Seed set (%) | 74.00 | 48.00 | 98.88 | 83.88 | 88.69 |
| | Mean ±*SE* | 153.00 ±56.48b | 95.25 ±27.30c | 148.75 ±3.64a | 211.25 ±22.16a | 231.50 ±15.84a |
| Emasculated, open | Seed set (%) | 42.00 | 20.00 | 24.38 | 18.50 | 18.65 |
| | Mean ±*SE* | 78.25 ±11.66c | 44.75 ±2.10d | 29.75 ±2.21c | 43.75 ±8.41b | 35.25 ±2.09c |
| Emasculated, bagged | Seed set (%) | 00.00 | 00.00 | 00.00 | 00.00 | 00.00 |
| | Mean ±*SE* | 00.00 ±0.00d | 00.00 ±0.00e | 00.00 ±0.00c | 00.00 ±0.00b | 00.00 ±0.00d |

Means in the same column followed by the same letter are not significantly different at $p = 0.05$.

## Insect visitor behavior

All insects observed visited each of the *Ocimum* variant studied. Insect visits were observed as early as 7 a.m. to 6 p.m. The frequency of insects' visits increased as soon as the flowers started opening between 7 a.m. and 2 p.m. The bees observed visiting the *Ocimum* species are shown in (Tab. 3), and include: *Apis mellifera* Linn., *Amegilla cingulata* Fabricius, *Dactylurina staudingeri* Gribido, *Lasioglossum* Curtis sp., and *Xylocopa calens* Lepeletier. The wasps observed visiting the *Ocimum* species include: *Vespula* Thomsom sp., *Pseudomasaris* Ashmead sp., and *Polybioides tabidum* Fabricius. Other insects observed on the *Ocimum* species were the butterflies, dragonflies, hoverflies, and the housefly. The bees and wasps visited the flowers of the *Ocimum* species to collect either nectar or pollen grains while *Musca domestica* L. and the dragonflies were observed visiting leaves and racemes so they cannot be considered as flower visitors. The butterflies visited the flower to collect nectar. The *Ocimum* pollen grains were observed on the insects most often but sometimes they carried pollen grains of other plants along with that of the *Ocimum* species. All bees, with the exception of *Apis mellifera* regularly visited studied *Ocimum* species. All wasps: *Vespula* sp. and *Polybioides tabidum* visited the plants very regularly except *Pseudomasaris* which visits were rare. The dragonflies and *Musca domestica* were also rare. The butterflies, however, were frequent in their visit. On all the bees, pollen grains were observed on their legs and abdomen but *Dactylurina staudingeri* carried pollen on legs only. The wasps also carried pollen on their legs and abdomen, except *Polybioides tabidum* which carried pollen on mouthparts. The butterflies carried few pollen grains but the

**Tab. 3** The insect visitors observed on the *Ocimum* species studied.

| Insect | Plant part visited | Foraging type | Range of time spent(s) | Frequency | Region of pollen deposition |
|---|---|---|---|---|---|
| Bees | | | | | |
| *Lasioglossum* sp. | Flower | Nectar/pollen | 6–38 | Regular | Abdomen, legs, mouth |
| *Apis mellifera* | Flower | Nectar | 2–5 | Rare | Legs, abdomen |
| *Amegilla cingulata* | Flower | Nectar | 3–10 | Regular | Legs, abdomen |
| *Dactylurina staudingeri* | Flower | Pollen grains | 4–37 | Regular | Legs |
| *Xylocopa calens* | Flower | Nectar | 3–8 | Rare | Legs, abdomen |
| *Braunsapis* sp. | Flower | Pollen grains | 3–6 | Rare | Legs, abdomen |
| Flies | | | | | |
| Syrphidae | Flower | Nectar | 3–6 | Rare | Legs |
| *Musca domestica* | Leaf and racemose | - | 3–23 | Rare | - |
| Wasps | | | | | |
| *Vespula* sp. | Flower | Nectar | 2–12 | Regular | Legs, abdomen |
| *Pseudomasaris* sp. | Flower | Nectar/pollen | 1–8 | Rare | Legs, abdomen |
| *Polybioides tabidum* | Flower | Nectar | 2–7 | Regular | Mouth |
| Others | | | | | |
| Butterfly (Lepidoptera) | Flower and leaves | Nectar | 14–62 | Regular | - |
| Dragon flies (Odonata) | Leaf and racemose | - | 11–82 | Rare | - |

dragonflies and *Musca domestica* were not observed to carry any pollen. Compared to other floral visitors, the bees were observed to carry more pollen grains due to their hairy body. The insects contacted pollen grains when they tried to feed on the nectar or pollen. The time spent on each flower visit by the floral visitors is indicated in Tab. 3. Insect individuals that feed on nectar alone spent less time on a flower than those that feed on pollen.

## Discussion

The seed set observed in the emasculated open treatments and lack of seed production in the emasculated bagged treatments of all three studied *Ocimum* species indicated that the insects that visited the flowers in the open treatments enhanced fruit and seed production. Also the production of higher fruit and seed sets in the open treatments when compared to the corresponding bagged treatments showed that insect visit enhanced fruit and seed production. Insects such as bees, wasp and hoverflies have been reported to play important functional roles as pollinators which facilitate fruit production in *Ocimum* species [19]. The production of high fruit and seed sets in the spontaneous self-pollination bagged treatments of the two *O. canum* variants and *O. basilicum* '$b_1$' indicated that their pollination occurred through autogamy. Autogamy in *O. canum* has been reported to be optional as it could also reproduce by cross-fertilization [20]. It has also been reported that *O. canum* is predominantly allogamous due to formation of smaller number of fruits from manual selfing and spontaneous selfing [21]. The low fruit and seed in the bagged spontaneous self-pollination treatment but significantly higher in the open treatment of *O. basilicum* '$b_2$' and *O. americanum* indicated that these two species showed out-crossing features and more flowers were pollinated by insects in the open treatment. *Ocimum basilicum* and *O. selloi* Benth have been reported to exhibits mixed reproduction system via out-crossing and autogamy [16]. A combination of protandrous condition (a mechanism promoting out-crossing), annual habit, herbaceous nature, more conspicuous floral parts and bee pollination in the Basilicum group which includes three studied *Ocimum* species have been considered as advanced characteristics of the genus [22]. The mixed mating system indicated a great reproductive versatility of these species which might have contributed to the high genetic variability in the genus, through intraspecific and interspecific hybridization. According to Darrah [13] the genus *Ocimum* is characterized by a great variability of both morphological features and chemical composition because cross-pollination has created a large number of species and subspecies. This might also be responsible for the variability in the chromosome number of the variants of *O. canum* and *O. basilicum* used in this study.

This study showed that successful pollination is marked by the corolla abscission (dropping) in all the three *Ocimum* species. Corolla abscission was observed to occur after successful pollination in other species of the family Lamiaceae [23] and was also directly related to pollination efficiency [24,25]. The corolla of the non-pollinated flowers remains intact for 4–5 days which still attract insects to effect pollination. The withering of corolla in *Ocimum* species is common in plants with many flowers that pollinators failed to visit [25]. According to Whitten [24] and Cruden [25] variation in flower longevity, marked by abscission (dropping) of the corolla, indicated an association with pollinating insects visit. This is also confirmed in the five variants of *Ocimum* species studied here. In the case of already pollinated flowers, post-pollination abscission increases the probability of visits to the remaining (non-pollinated) flowers on the plant.

Result of anther dehiscence indicated that the five *Ocimum* variants varied in their period of anther dehiscence. In *O. canum* '$c_1$' and the two variants of *O. basilicum* anther dehisces before anthesis while in *O. canum* '$c_2$', *O. americanum* anther dehisces occurred after anthesis takes place. Anther dehiscence has been reported to occur before anthesis in *O. canum*, *O. basilicum*, *O. americanum*, and *O. officinalis* [11,15,21,26]. Pollination in *O. basilicum* and *O. americanum* has been reported to occur mainly in post-anthesis which coincides with period of intense insect visitation which favored out-crossing because the insect carried pollen from flower to flower [14,16,27]. The

period of anthesis coincided with the peak of insect visit in this study which might be due to the peak of nectar production and availability of pollen. According to Kulloli et al. [28] nectar secretion occurred 2 hours before flower opened and increased soon after the flower opened which continued till early morning in members of the Lamiaceae. This also coincided with the time of maximum pollinator activity.

Insects foraging for both pollen and nectar of studied plants could affect pollination. *Ocimum* species have been reported to rely on insects for pollination [11]. Bees foraging on pollen grains spent more time using their legs to rub against the anther and stigma in a bid to harvest pollen. But those foraging on nectar spent lesser time on flower but their landing position on the lower lip of the petal made their ventral surface to contact the stigma and anther which enhanced pollination. Longer visits were reported to correlate positively with pollination efficiency because it allowed more pollen collection from flowers [29,30]. Other factors such as larger body size, navigation pattern might also effect pollination [31]. Insects that spent less time on a flower were able to visit and navigate more flowers. This might increase the number of flowers pollinated during a single visit to an inflorescence. However, the tendency of pollinators to visit several flowers on a single plant increases opportunity of geitonogamy and self-pollination rate [32–35]. The repeated visits by various insects to our study plants to collect nectar and pollen suggests that they produced enough reward to the visitors which may enhance pollination. Nectar production is the most common floral reward furnished by animal pollinated plants to their mutualistic partners [36].

## Conclusion

The two *O. canum* variants and *O. basilicum* '$b_1$' are primarily adapted to reproduction by autogamy while *O. basilicum* '$b_2$' and *O. americanum* exhibited mixed mating system via out-crossing and autogamy. Insect pollination in the studied plants enhanced fruit and seed production.

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## Porównanie mechanizmów reprodukcji trzech gatunków z rodzaju *Ocimum* L. (Lamiaceae)

### Streszczenie

Gatunki z rodzaju *Ocimum*, zależnie od występowania i odmiany, reprezentują różne systemy reprodukcyjne. W pracy badano systemy reprodukcyjne trzech gatunków *Ocimum canum* Sims., *O. basilicum* L. i *O. americanum* L. Badania prowadzono w Nigerii. Zastosowano następujące sposoby zapylania: zapylanie swobodne (z udziałem owadów zapylających) oraz pod izolatorem (dopylanie pyłkiem własnym, spontaniczne samozapylanie z usuwaniem pylników). Wszystkie gatunki produkowały więcej owoców i nasion w warunkach swobodnego dostępu owadów do kwiatów. Kwiaty *O. canum* i *O. basilicum* '$b_1$' swobodnie dostępne dla owadów oraz spontanicznie samozapylane pod izolatorami produkowały najwięcej owoców i nasion. *Ocimum basilicum* '$b_2$' oraz *O. americanum* produkowały najwięcej owoców i nasion podczas samozapylenia z kwiatów nieizolowanych, istotnie mniej owoców i nasion powstawało z kwiatów izolowanych od owadów. Mniej owoców uzyskiwano z kwiatów nieizolowanych, z których usuwano pylniki. Kwiaty *Ocimum*, izolowane bez pylników, nie wiązały nasion i nie tworzyły owoców. Owady odwiedzające kwiaty badanych gatunków z rodzaju *Ocimum* (pszczoły, osy, motyle) korzystały z nektaru lub pyłku. *Ocimum canum* i *O. basilicum* '$b_1$' rozmnażają się głównie autogamicznie. *Ocimum basilicum* '$b_2$' oraz *O. americanum* wykazują mieszane systemy reprodukcyjne (autogamia oraz zapylenie krzyżowe). W przypadku wszystkich badanych gatunków z rodzaju *Ocimum*, owady wspomagają zapylenie, powodując wzrost produkcji owoców i wiązanych nasion.