Effect of Pollination Methods on Onion (*Allium cepa* L.) Seed Yield and Quality

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Abstract

Onion (Allium cepa L.) is a strongly protandrous crop that requires cross-pollination by insects and different agents for seed production. This experiment was conducted in the years 2021 and 2022 with the objective to investigate the effect of pollination methods on onion seed yields and seed quality attributes. One onion variety (Naffis) and four pollination methods were compared during two production seasons. RCBD and CRD designs for field and laboratory experiments were used respectively. The treatments were arranged with factorial in four replications. The results of analysis showed that onion seed yields, analytical purity, thousand seed weight, germination percentage, seedlings length (shoot and root), seedling dry weight, seedling vigor index-I and II were significantly affected by main effects of treatments and their interactions. Mean seed yield of variety produced with honeybee pollination methods showed highly significant ($P \leq 0.01$) difference than seed produced in open field pollinated by different pollination agents. Among analytical purity components pure seed, and inert matter showed highly significant ($P \leq 0.01$) differences between production seasons and pollination methods. Highly significant ($P \leq 0.01$) interaction effects were found with year of production and pollination methods for germination percentage. Seeds produced with honeybee pollination showed highly significant ($P \leq 0.01$) differences regarding seedling shoot and root lengths, seedling dry weight and for seedling vigor index-I and -II. The results suggested that pollination of onion seeds by honeybee is better for production of high seed yields and analytical purity and physiological (Germination and vigor induces) seeds quality.

Keywords: Onion; *Allium cepa*; Pollination methods; Quality seed; Seed vigor indices.

Introduction

Onion (*Allium cepa* L.) is a major bulb crop among the cultivated vegetable crops with global importance. Onion is an important source of vegetables, and its seed is a source of condiments (Baswana, 1984). Among the horticultural crops in Ethiopia, root crops are higher in area coverage with 307,295.80 ha as compared to vegetables and fruit trees. Onion is a popular root crop and is widely cultivated across five regions in Ethiopia (Oromia, Amhara, Somali, Tigray and SNNPR). In 2021, onion was cultivated on 38,952.58 ha with the total production of 562,168.153 tons with the average productivity of 14.432 tons/ha (CSA, 2021). In India, the yields of onion dry bulb is 12.5 t/ha which is much lower than the USA at 41.12 t/ha (FAO, 2002), the higher productivity in USA is due to growing long day hybrid varieties.

The floral biology, pollinators and their abundance, foraging behavior, pollination efficiency and mode of onion pollination are among many factors that can affect seed yields and quality. Onion crop is strongly protandrous; Self-pollination is largely absent which depends upon insects for cross pollination (Baswana, 1984). Onion umbels are visited by honey bees, small syrphid flies, bumble bees, halictid bees, drone flies, butterflies and insects of minor importance with respect to pollination (Sajjad *et al*., 2008).

Even though onion seed production is carried out by formal, intermediary and informal seed sectors, the quality of seed used for onion production remains a major challenge. To get maximum onion seed yield and high-quality seed, onion seed producers are facing many problems. In Ethiopia, the average bulb yields varied from 30 to 40 tons per hectare in the research fields whereas it is by far below these in the farmer's fields. The average true seed yields of onion varied from 1 to 1.6 tons per hectare in the research fields whereas the farmer's managed fields are by far low due to several factors such as variety, seed quality, planting season, pollinating agents, planting space and etc. Depending on several factors (e.g., variety, date of sowing and planting space) the average seed yield may vary from 0.828 to 1.45 tons/ha (Aminpour and Mortzavi 2004).

Onion does not produce good quality seed in the absence of abundant pollinators (Chandel *et. al*., 2004) and loss of bulb yield may be as high as 28% after three consecutive generations of inbreeding. Kumar *et al*. (1989) reported that in India the greater onion seed set, seed yield and better seed germination was from plots caged with bees, than plots caged without bees and open plots with estimated seed yields of 275, 73 and 97 kg/ha, respectively. Honey bees are the most important pollinators for onion crop because the duration of pollinator visits to a flower is correlated positively to pollination rate, which are also correlated to the number of seeds produced, their weight and their seedling lengths. Yucel and Duman (2005) reported that seed yield per bulb was higher in open plots (5.74 g/flower) compared to 1.29 g/flower in caged plots. Ruszkowaski and Bilinski (1984) found that the yield of hybrid seed in cages pollinated by honey bees, bumble bees (*Bombus terrestris* or *B. ruderarius*) and *Megachile rotundata* was equivalent to 301, 295 and 47 kg/ha, respectively in Poland.

The limited availability and low production of onion seed yields could be due to reduced pollination by pollination agents, leading to use of genetically and physically poor-quality seeds from informal sources. Factors such as continuous use of pesticides and decline in natural habitats to some extents are responsible for decreasing the availability of natural insect pollinators (Saeed *et al.*, 2008). Since onion flowers are not capable of self-pollination, out-crossing becomes more critical due to the protandrous nature of the onion flowers plant (Muller, 1883). The amount of out-crossing may vary from 8 to 71% under different conditions (Van der Meer and Van Bennekom, 1968). Due to the high amount of out-crossing, the probability of onion seed quality contamination is high. Therefore, the spatial isolation distance of one variety from the others should be maintained for each seed class based on national seed quality standards. Due to shortage of budget allocated for early generation seed multiplication and limited farm lands of research centers multiplying many varieties at the same time was among the factors affecting onion seed quality and quantity. Low seed yield per hectare while producing EGS was also critical challenge for onion seeds. The main objective of the current study was to determine the effects of various pollination methods on onion seed yields and seed quality attributes.

Materials and Methods

Description of the study sites

The experiment was conducted at Seed Research and Quality Control Laboratory of Melkassa Agricultural Research Center from 2021 to 2022, Melkassa, Ethiopia. It is located in Adama wereda in East Shewa Zone with the longitude of 8^{o}25'17" N and 39^{o}19'56" E, and altitude of 1550 m.a.s.l. in the central rift valley of Ethiopia. The distance of the site from the capital city, Addis Ababa is 117 kilometers in the eastern part of the country. It receives a mean annual rainfall of 763 mm with erratic distribution with peaks occurring in July and August in a mono-modal pattern. The recent annual averages of minimum and maximum temperatures in the study area are 12.5 ^{o}C and 28.6 ^{o}C, respectively.

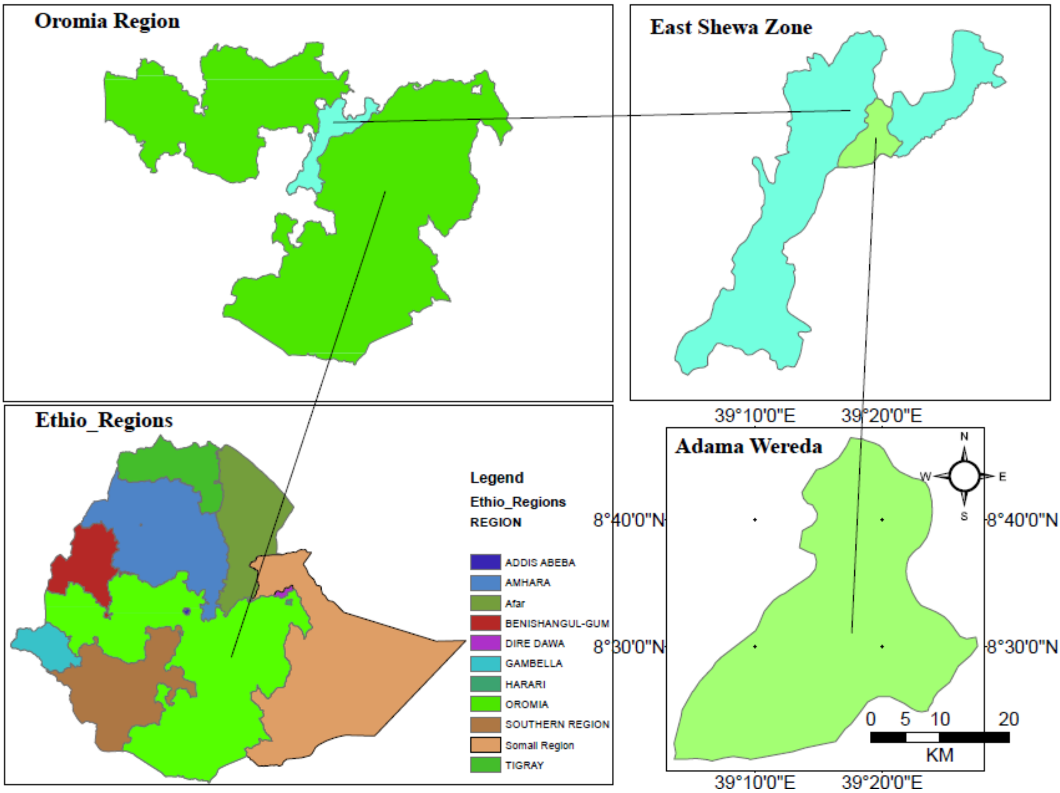

Figure 1. Representative map of study area in Ethiopia

Meteorological data

Temperature: Temperature, relative humidity and rainfall were the most significant factors that affect onion true and bulb seeds quantity and quality during production and storage. The experiment was carried out in the cages and open field plots. The average temperature at which onion seed production experiment carried out was ambient temperature that recorded from January to September for two years. In 2021, the average temperature was ranged from 13.97 to 29.51 ˚C for the ambient environmental system, while, the recorded minimum and maximum temperature was 9.93 and 32.59 ˚C respectively (Table 1). In 2022, the average temperature was ranged from 14.15 to 29.89 ˚C for the ambient environmental condition, while, the recorded minimum and maximum temperature was 9.20 and 34.28 ˚C respectively (Table 2).

Relative humidity: The average relative humidity of ambient environmental condition was recorded for two years. In 2021, the relative humidity fluctuated from 40.10 to 70.13 % for the ambient environmental condition (Table 1) whereas in 2022, the relative humidity fluctuated from 40.16 to 69.53% for the ambient environmental system (Table 2).

Rainfall: The amount of rainfall in the area where the onion seed production experiment carried out was recorded from January to September for two years. In 2021, the recorded average rainfall was 2.51 mm, while, the minimum and maximum rainfall were 0 and 10.85 mm respectively (Table 1). In 2022, the recorded average rainfall was 2.42 mm, while, the minimum and maximum rainfall were 0 and 11.64 mm respectively (Table 2). However, the onion seed production experiment was done using full and supplementary irrigation (rainfall exist) supplied.

Table 1. Meteorological data for 2021 cropping season by months

List of months	Monthly minimum mean temperature (^{0}C)	Monthly maximum mean temperature (^{0}C)	Monthly mean relative humidity (%)	Monthly mean rainfall (mm)
January	9.93	28.44	45.23	0.00
February	12.35	28.52	50.21	1.35
March	15.47	32.59	40.10	0.00
April	15.73	32.42	42.60	2.54
May	16.71	31.42	54.55	0.98
June	17.12	32.50	49.80	1.49
July	16.44	25.98	70.13	6.10
August	15.95	27.82	66.32	10.85
September	15.43	27.91	68.87	4.63
October	11.95	28.56	60.65	1.78
November	10.57	29.36	47.40	0.43
December	10.00	28.57	48.71	0.00
Average	**13.97**	**29.51**	**53.71**	**2.51**

Table 2. Meteorological data for 2022 cropping season by months

List of months	Monthly minimum mean temperature (^{0}C)	Monthly maximum mean temperature (^{0}C)	Monthly mean relative humidity (%)	Monthly mean rainfall (mm)
January	12.74	29.47	51.13	1.26
February	12.33	30.70	47.21	0.00
March	15.96	31.73	44.32	0.99
April	16.20	33.50	41.00	1.45
May	15.23	34.28	40.16	0.00
June	17.03	31.59	51.50	1.32
July	16.65	26.85	64.68	6.55
August	16.43	27.23	68.68	4.09
September	15.51	27.53	69.53	11.64
October	13.05	28.45	58.06	1.70
November	9.20	28.58	54.63	0.00
December	9.48	28.81	63.00	0.00
Average	**14.15**	**29.89**	**54.49**	**2.42**

Experimental material

Popular onion variety namely naffis that released from MARC was used in this experiment. The experiment was conducted in the 2021 and 2022 years. The pre basic seed of naffis variety was obtained from early generation seeds produced by technology multiplication and seed research department of Melkassa Agricultural

Research Center. The experiment was used from the onion bulb seeds produced during 2020 and 2021 cropping seasons.

Experimental design and treatments

The treatments of field experiment were laid out in randomized complete block design arranged in 2x4 factorial combinations with four replications. One common onion variety (Naffis) was used with two categories of treatments (plots with cages and in open fields). Plots with cages were pollinated by honeybee, shaking by hand and other pollination factors. Plots without cages in open fields were pollinated by different pollination agents. Bulbs were planted in four directions (North, South, East and West) surrounding caged plots. Each year the experimental plots have followed the same pollination methods.

Experimental procedures

Field experiments

Caged plots: Each year, 100 bulbs with medium size of naffis variety was selected and planted on each plot. Bulbs were planted with spacing of 20 cm between bulbs and 50 cm between rows. For each plot a wooden structure was constructed around the plots and covered with insect proof cages. The total area of each plot was $20m^2$ (4m x 5m) whereas the bulbs were planted only on $12m^2$ (3m x 4m) net area with the roof height of 2.5m (**Fig. 2**). From the 12 plots constructed with wooden structures, four plots were pollinated by honey bees, four plots were pollinated by hand shaking and four plots were left for self-pollination without any pollination agents.

Open plots: Four open plots were planted with the same variety in open air fields on the net area of 12 m^2 each plot with a distance of 1.5 m far and surrounding main field (caged plots) in four directions (N, S, E and W) and the caged plots. Bulbs of this onion variety were planted in open fields to check whether early generation seed of many onion varieties can be produced in the cages and open fields at the same time without affecting the seed yields and other seed quality parameters. All agronomic practices were applied as recommended for onion seed production.

Onion pollination methods

Honey bee pollination in cages, four colonies of honeybees i.e. one colony for each plot was used. Honey bees with hives were transferred from the animal science research program to the area where these trials were conducted and kept for 30 days to break dormancy. After 50% of onion seeds have flowered, pollination with the honeybees was started. After honey bees colonies have placed in the cages, the gates were closed immediately. Each honeybee colony was kept for one day (24hrs) in the cages and removed out of the cages for 30 days. The honeybees' colonies were managed in the night when the movement was rare or stopped. Since the onion flowers were not enough to nourish the bee colonies, additional feeds (sugar and field pea flour) were purchased from local markets. Sugar was mixed with a little amount of water and field pea flour prepared by human and kept in the dish for honeybee feeds. A total of 8 kg of sugar and 6 kg of field pea flour were used by the four colonies of honeybees during pollination periods of 30 days.

Hand pollination method by human was started after onion seeds flowering have reached around 50%. For pollination of onion flowers by hand shake, two field technicians were handled the ropes by both right and left hands and shook it to pollinate the flowers. The ropes were captured below the umbel on the flower stalks. The workers shook the onion stalks slowly to make the plants contact each other for about 8-10 minute per row (**Fig.** C). After they have finished shaking the doors were closed immediately. Self-pollination method was used by planting onion bulbs after flowers in the cages. After onion bulbs have planted, the doors were closed till the seed was physiologically matured enough for harvest. After seeds have physiologically matured enough, the doors were opened and harvesting of umbels was done. A pollination method by various pollinators in the open field was also used. Onion bulbs were planted in the four open plots with the same variety in the four directions and left open to be pollinated by various pollination agents.

Harvesting and cleaning seeds

Harvesting started after 20-30 % of black seeds have visibly exposed in the umbels, by cutting the umbels with 5 cm stalk and was done for 3 to 4 times in 2 to 3 days intervals to reduce seed losses. Harvested umbels were placed separately in the polypropylene woven bags and taken to the warehouse. The seeds were dried with the umbels in the morning and afternoon sunlight by spreading on the canvas. After drying, the umbels were threshed manually by stick beating to separate the seeds from straw and cleaned by winnowing in wind blowing. The cleaned seeds were also soaked in clean water to separate the floating seeds that assumed to be of poor quality seeds. The pure (sinked) seeds were re-dried for 5 to 7 days by exposing in the morning and noon sun light and at night by spreading the seeds on canvas in the store room till the maximum moisture contents of the seeds reached 9%. After all harvested seeds have cleaned and dried; seed samples taken from each plot were divided into two equal parts using a sensitive digital balance for purity analysis.

Laboratory test for seed quality

The physical and physiological (germination and vigor) seed quality tests were conducted using 80 grams seeds samples which were withdrawn from each treatment and replication as composite samples thoroughly mixed primary samples and submitted working samples to laboratory. The submitted seed samples were used for seed quality analysis in the seed research and internal quality control laboratory at MARC. The seed quality test parameters such as analytical purity, 1000-seed weight, germination capacity, shoot length, root length, seedlings dry weight, vigor index-I (VGI-I) and vigor index-II (VGI-II) were carried out following procedures set for each parameter.

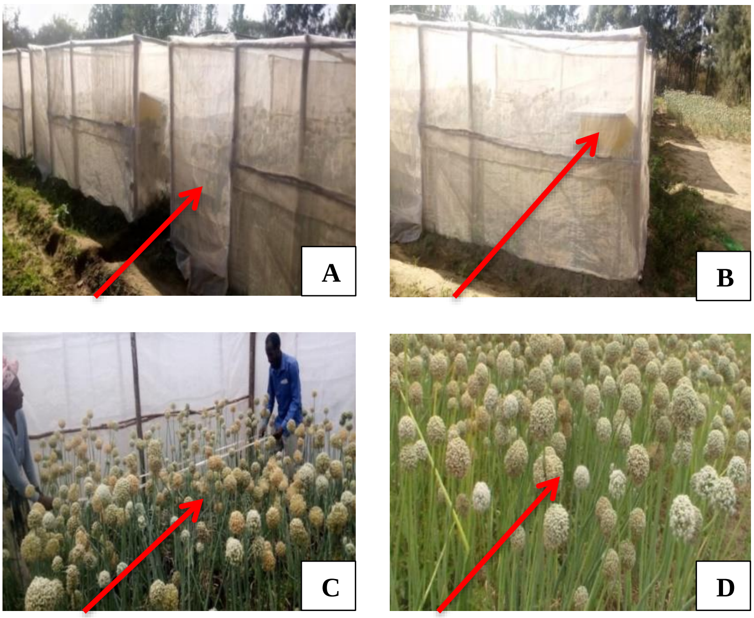

Figure 2. Methods of onion seed pollination in insect proof cages and open plots (A to D)

Data collection

Analytical purity test: After post-harvest seed processing, eighty grams (80 g) of open pollinated onion seed samples were taken from each treatment and variety and submitted to the laboratory for seed quality analysis. All 80 g of the submitted sample was subjected to seed quality tests as working sample to laboratory. Each working sample was divided into two replicates (40 g each) for physical purity analysis. The components were separated into pure seed, inert matter and other seeds. Each component was weighed using an analytical digital balance. The percentage composition of the seed lot was calculated based on the weight of each component (ISTA, 2016).

$$\text{Purity (\%)} = \frac{\text{Weight of pure seed}}{\text{Weight of total sample}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Thousand seed weight: Thousand seed weight of each sample was determined by counting the entire working sample by seed counter machine. After seeds have counted, the samples were weighed in grams to the same number of decimal places used by KERN balance or sensitive balance as in the purity analysis. The whole working samples were put through the machine and the number of seeds on the indicator was read. The weights of 1000 seeds were calculated from the weight of the whole working samples (ISTA, 2016).

Physiological seed quality tests

The physiological seed quality test is the viability, germination and vigor of the seed which determines the germination and subsequent seedling emergence rate and crop establishment in the field as well as the storage potential of the seeds lots.

Germination percentage: A germination test was carried out for all seed samples collected from each treatment produced in the insect proof cages and open field conditions. The pure seed, separated during purity analysis was considered for this test. Four hundred onion seeds were divided into four replicates of one hundred seeds replication each and planted in germination paper substrata at ambient room temperature. The first count was made on the 6th day whereas the final count was carried out on the 12th day (ISTA 2016). On the final count of germination test, seedlings were evaluated into normal seedlings, abnormal seedlings, dead seeds, and infected/ fresh un-germinated seeds components. For each seed sample, the result was expressed as the mean percentage of normal seedlings. To calculate the percentage of normal seedlings, the following equation was used (ISTA, 2016).

$$\text{Germination } (\%) = \frac{\text{Number of normal seedlings}}{\text{Total number of seed sown}} \times 100 \text{ .... } (2)$$

Seed vigor test

Seed vigor could be determined by using different seed quality test parameters that were carried out in the laboratory. The following tests were used to determine the seed vigor.

Seedlings shoot and root lengths: The seedling shoot and root lengths were measured by ruler immediately after the final count was made in the standard germination tests. Ten normal seedlings were randomly taken from each replicate. The shoot length was measured from the point of attachment to the cotyledon to the tip of the seedling. Similarly, the root length was measured from the point of attachment to the cotyledon to the tip of the root. The average shoot and root lengths were computed separately by dividing the total shoot and root lengths by the total number of normal seedlings measured (Fiala, 1987).

Seedlings dry weights: The seedling's dry weight was measured after the final count of the standard germination test. Ten seedlings were randomly taken from each replicate and appropriately placed in the envelope and put in an oven dry and dried at 80 °C for 24 hours. After seedlings have been dried, the dry matter was weighed to the gram and the average seedlings dry weight was calculated.

Vigour indices: The seedling vigour index was calculated for each sample as per Abdul Baki and Anderson (1973) and expressed in number by using formula below. Seedling vigour index-I was calculated by multiplying the standard germination with the average sum of shoot length and root lengths after 12 days of seedlings incubation, while Seedling vigour index-II was calculated by multiplying the standard germination percentage with mean of seedling dry weight (dried at temperature of 80±1 ºC for 24 hours) according to (ISTA, 2016). The formula for these parameters: VGI-I = Standard germination × mean of seedling lengths (Average mean of shoots + average mean of roots lengths) whereas VGI-II= Standard germination × mean of seedling dry weight.

Data analyses

Differences in quantity and quality of onion seed production were analyzed by two-way full factorial ANOVA (Year*treatments (pollination methods)*variety). When ANOVA indicated a significant effect at 1% and 5% levels, Fisher's Protected LSD test was used to determine significance between mean values. Statistix-8 User Guide for plant materials program, version 2.0 was used for all statistical analyses.

Results and Discussion

The analysis of variance showed that cropping season had significantly influenced seed yields whereas pollination method had highly significant influence on onion seed yields (Table 3). Cropping season had significant influence on most seed quality parameters of pure seeds, inert matter, thousand seed weight, seedlings dry weight, shoot length, and vigor index-II. A non-significant effect observed among cropping seasons on some seed quality parameters such as standard germination, root length and vigor index-I. Highly significant differences were observed among pollination methods regarding all tested seed quality parameters of pure seeds, inert matter, thousand seed weight, seedlings dry weight, standard germination, shoot length, root length, and vigor index-I and II. Besides, production seasons and pollination methods interacted to influence significantly pure seeds, inert matter, standard germination, seedling dry weight and vigor index-II. This might be due to seeds pollinated by various methods, genotypes and environmental factors that varied physiological and physical seed quality parameters, and seed yields.

This indicates that, Seed yields and physiological seed quality parameters are the result of changes among the cropping season and methods of pollination within treatments and their interaction that increase yields and other quality parameters. Van der Meer and van Bennekom (1968) reported only 9 percent self-fertilization in onion crop which corroborated with the present finding of low seed set in self-pollination plot. Increase in seed set and germination with insect pollination have been reported in a number of instances. Seed production in onion was increased to 8 to 10 times by using bees (Sanduleac, 1961). Kumar *et al.* (1989) calculated the estimated seed yield as 275 kg/ha in bee pollinated area compared with 98 and 3 kg/ha in open and self-pollination in onion, respectively. Ahmed and Abdalla (1984), Yucel and Duman (2005) showed that honey bee pollination provides seeds to be more coarse and filled. This contention seems true as average seedling length was highest in Bee pollination (11.99 cm) followed by 10.73 and 10.03 cm in Open-pollination + Hand-pollination and Open-pollination, respectively.

Onion seed yields

Different onion varieties may have different seed yield potential in terms of bulbs and true seed yield. Yields of onion true seeds can be affected by many factors among which pollination methods are the one. Mean seed yields of naffis variety in insect proof cages pollinated by honeybee showed higher seed yields than mean seed yield of naffis variety produced in open fields (Table 3). Moreover, highly significant ($P \leq 0.01$) difference was observed between honeybee pollination and other pollination methods caged in insect proof and open field plots regarding onion seed yields. Naffis variety produced by self-pollination method in insect proof cages showed lower seed yields than the same variety produced by honeybee pollination and hand pollination method in insect proof cages and open fields. Similar studies indicated that under honeybee pollinated field the increase in seed production ranged from 175 (Deodikar and Suryanaryana, 1972) to 1000 percent (Singh and Dharamwal, 1970). Seed yield difference among treatments showed that the seed produced in 2021 season showed significant ($P \leq 0.05$) difference whereas highly significant ($P \leq 0.01$) difference was observed between treatments produced in 2022 cropping season (Table 3). This might be due to environmental factors, pollination methods and genetic potential of the variety used in this experiment. The temperatures required for onion seeds production have a great influence on seed yield and quality where optimum temperature is crucial during flower initiation and stalk development. Study result carried out by Khokhar (2009) on the effects of temperature on inflorescence initiation in onion revealed that the optimum temperature for flowering is in a range between 5˚C to 13˚C.

There was no interaction effects observed between "production season*pollination methods" regarding onion seed yields (Table 4). There was significant ($P \leq 0.05$) differences observed between production seasons in terms of seed yields (Table 3). The onion seed yield variation in different pollination methods might be due to high flower abortion and consequently low seed set in self and hand pollinated onion seeds (Table 3). Lower seed yields produced by hand shaking than seeds produced by various pollinating agents and honeybee might be due to dropping of some pollen from umbels while shaking of umbels to contact each other. Abortion of flowers of onion seeds pollinated by self-pollination method in insect proof cages was higher than other pollination methods. This might be due to shortage of cross pollinating agents. This finding of low seed set in self and hand pollination methods corroborated with the study reported by Van der Meer and Van Bennekom (1968) where there is only 9 percent of self-pollination in onion seed crop. In India, Kumar *et al*. (1989) found that greater onion seed set, seed yield and better seed germination of plots caged with bees, than plots caged without bees and open field plots and the estimated seed yields were 275, 73 and 97 kg/ha, respectively. According to Chandel *et al*. (2004), induced bee pollination increased seed yield by 2.5 times and produced on an average 971 seeds per umbel compared to 406 in the control. Yucel and Duman (2005) reported that seed yield per bulb was higher in open field plots (5.74 g/flower) as compared to 1.29 g/flower in caged plots. Woyke (1981) observed that numbers and weight of onion seeds from self-pollination were very low compared with yields in open fields provided with honeybee colonies.

Table 3. Means of onion seed yields by years and treatments

Years	Seed yields (kg/ha)
2021	638.02a
2022	505.73b
LSD (0.05)	**128.45***
CV	**30.55**
Treatments	
SP	264.27c
HP	385.21c
HBP	910.42a
VPA	727.6b
LSD (0.05)	**181.65****
CV	**30.55**

SP=Self-pollinated, HP=Human pollinated,
HBP=Honeybee pollinated, VPA=Various pollinated agents

Table 4. Means of onion seed yields by 'year*treatment' interaction effects

Years	Treatments	Seed yields (kg/ha)
2021	SP	318.75de
2021	HP	507.29cd
2021	HBP	879.16a
2021	VPA	846.88ab
Mean		**638.02**
2022	SP	209.79e
2022	HP	263.13de
2022	HBP	941.66a
2022	VPA	608.33bc
Mean		**505.73**
LSD (0.05)		**256.90ns**
CV		**30.55**

SP=Self-pollinated, HP=Human pollinated, HBP=Honeybee pollinated, VPA= Various pollinated agents.

Seed quality test

Analytical purity: Using different pollination methods in onion seed production within two consecutive seasons recorded highly significant ($P \leq 0.01$) differences regarding pure seeds and inert matter percentages. In the first season compared with the Ethiopian national seed quality standard all treatments satisfied with the minimum seed quality standard set for pure seed (97%) of basic seeds class. Moreover, in the second season honeybee pollinated in the insect proof cages and open pollinated in the open airfield satisfied the minimum national seed quality standard for pure seeds whereas hand pollinated and self-pollinated onion seeds in insect proof cages caused not fulfill the national seed quality standard in the country. The low percentage of pure seeds was probably due to the immature and partially unfilled seeds, which simply identified by magnifying lens on seed purity board. There were highly significant ($P \leq 0.01$) differences observed between seasons and treatments (pollination methods) in terms of pure seeds percentages (Tables 5 and 6).

Sinking onion seeds in clean water enable to separate light weight seeds which are immature and partially filled seeds floated on the water. In seeds pollinated by self and hand pollination methods floaters seeds were higher than the other two pollination methods which was included in inert matter during purity analysis. In Colorado (USA), a similar study was reported by Brewer (1974) revealed that both the number and weight of mature seeds per melon were significantly higher from plots visited by bees than those where bees were excluded. Seeds which were settled expected to be good seed and after drying to the optimum moisture could be used for planting purpose. The interaction effects of production seasons and pollination methods showed highly significant ($P \leq 0.01$) difference observed regarding percentage of pure seeds (Table 7). This might be due to environmental factors and pollination methods.

Thousand seed weight

Honeybee pollinated seed produced in insect proof cages showed higher 1000 seed weight compared with seeds produced by hand and self-pollinations of the same variety produced in insect proof cages (naffis) and open field plots (Table 6) and **Fig. 2** (A, B, C and D). There was highly significant ($P \leq 0.01$) difference observed between years of production regarding 1000 seed weight (Table 6). The years of production in 2021 and 2022 gave 4.27 and 3.91 grams respectively. There was high significant ($P \leq 0.05$) difference observed between pollination methods regarding 1000 seeds weight (Table 6). The interaction effects of production year by pollination method regarding 1000 seed weight were not indicated significant (Table 7). A similar result work was reported by Hussein *et al*. (2013), who stated that the number of seeds and weights of 1000 seeds in treatment without pollinating insect were significantly less than that of the free pollinated treatment. The findings of this study are in line with work of others elsewhere (Jaboñski *et al*., 1982; Rao and Lazar 1983; Ahmad *et al*., 2003; Tolon and Duman 2003; Kumar *et al*., 1989), who confirmed that the presence of insects during flowering period has substantial impact on onion seed setting.

Standard Germination

Seed germination results revealed that the seeds produced with honeybee pollination agent in insect proof cages had higher germination capacity (91.04%) compared with the seeds produced by hand and self-pollinations in insect proof cages (83.96 and 80.78%) respectively and seeds produced by various pollination agents in open fields (84.98%). Highly significant ($P \leq 0.01$) differences was observed between pollination methods regarding germination percentage (Table 4). In this study there was no significant effect observed between years of production regarding germination percentage. However, the interaction effects between year of production and pollination method on germination percentage were highly significant ($P \leq 0.01$) (Table 7). This result is in agreement with earlier finding reported by Tolon and Duman (2003) who stated that germination capacity and energy are higher when onion flowers are pollinated by honey bees. Moreover, in this study the difference of germination capacity between pollination treatments was similar with other research works. For example, Chandel *et al*., (2004) reported that the difference in onion seed germination capacity derived from plantations where pollinating insects were introduced in relation to plantations was reached over 20% than from the insect absent plantation. The seeds from induced pollination field resulted in 90 percent germination compared to 69.5 percent germination from the control. A similar study reported by Chandel *et al*. (2004) who indicated that seeds from induced bee pollination showed maximum germination percentage as compared with control.

Vigor indices

There were highly significant differences observed among seedling shoot and root length (cm), seedling dry weight (g), vigor index-I and vigor indexes-II within various pollination methods. Seeds produced through honeybee pollination method in insect proof cages showed highly significant ($P \leq 0.01$) differences regarding seedling shoot and root length, seedling dry weights and hence seedling vigor index-I and seedling vigor index-II (Table 6). Both vigor index-I (VGI-I) and vigor index-II (VGI-II) showed highly significant ($P \leq 0.01$) differences between seeds produced under different pollination methods. However, no significant difference was observed for VGI-I whereas highly significant ($P \leq 0.01$) difference was observed for VGI-II among the years of production (Table 5). Significantly lower values of VGI-I (979.40) and VGI-II (2.72) were obtained from seeds produced in cages pollinated by self-pollination. This variation might be due to genetic potential of the variety, methods of pollination and other environmental factors. The findings of the current study agreed with, a study carried out on groundnut that revealed root length, shoot length, seedling dry weight, and seedling vigour indices differed significantly among varied groundnut varieties and seed sources (Mahesh, 2007). There were highly significant ($P \leq 0.01$) interaction effects of year of production and pollination methods on seedling dry weight and seedling vigor index-II whereas no significant interaction effects were observed on seedlings shoot and root lengths, and seedling vigor index-I (Table 7). A similar study revealed that seeds from induced bee pollination showed higher seed set (Prasad *et al*., 2000), maximum germination (Chandel *et al*., 2004), shoot and root length (Kalmath *et al*., 2004) compared to control. Onions do not produce high quality seeds if insects do not visit the flowers (Hussein *et al*., 2013). There was a high significant ($p \leq 0.05$) interaction effect of production year by pollination method regarding percentage of inert matter.

Table 5. Seed quality parameters (PS (%), IM (%), TSW (g), SG (%), SDW (g), SL (cm), RL (cm), VGI-I and VGI-II) by years.

Years	PS (%)	IM (%)	TSW (g)	SG (%)	SDW (g)	SL (cm)	RL (cm)	VGI-I	VGI-II
2021	98.84a	1.16b	4.27a	85.31a	0.03b	9.92a	2.70a	1077.10a	2.61b
2022	95.62b	4.38a	3.91b	85.06a	0.04a	9.59b	2.78a	1052.10a	3.32a
LSD(0.05)	**0.85****	**0.85****	**0.18****	**1.81^{ns}**	**0.001****	**0.30***	**0.16^{ns}**	**34.16^{ns}**	**0.12****
CV	**1.19**	**41.70**	**6.00**	**2.89**	**4.77**	**4.21**	**7.96**	**4.36**	**5.26**

Note: PS= Pure seeds, IM= Inert matter, TSW= Thousand seed weight, SG= Standard germination, SDW= Seedlings dry weight, SL= Shoot length, RL= Root length, VGI-I= Vigour index one, VGI-II= Vigour index two.
Means within a column followed by the same letter are not significantly different from each other at 5% Probability level based on LSD test.

Table 6. Seed quality parameters (PS (%), IM (%), TSW (g), SG (%), SDW (g), SL (cm), RL (cm), VGI-I and VGI-II) by treatments.

Treatments	PS (%)	IM (%)	TSW (g)	SG (%)	SDW (g)	SL (cm)	RL (cm)	VGI-I	VGI-II
SP	94.85b	5.16a	4.06ab	80.78c	0.035b	9.87a	2.47b	979.40c	2.72c
HP	96.01b	3.99a	4.22a	83.96b	0.037a	10.03a	3.02a	1095.40b	3.06b
HBP	99.57a	0.43b	4.27a	91.04a	0.036ab	10.11a	2.94a	1186.70a	3.28a
VPA	98.49a	1.51b	3.82b	84.98b	0.032c	9.01b	2.52b	997.00c	2.78c
LSD(0.05)	**1.20****	**1.20****	**0.26***	**2.56****	**0.002****	**0.43****	**0.23****	**48.31****	**0.16****
CV	**1.19**	**41.70**	**6.00**	**2.89**	**4.77**	**4.21**	**7.96**	**4.36**	**5.26**

Note: PS= Pure seeds, IM= Inert matter, TSW= Thousand seed weight, SG= Standard germination, SDW= Seedlings dry weight, SL= Shoot length, RL= Root length, VGI-I= Vigour index one, VGI-II= Vigour index two, SP=Self-pollinated, HP=Human pollinated, HBP=Honeybee pollinated, VPA= various pollinated agents.
Means within a column followed by the same letter are not significantly different from each other at 5% Probability level based on LSD test.

Table 7. Seed quality parameters (PS (%), IM (%), TSW (g), SG (%), SDW (g), SL (cm), RL (cm), VGI-I and VGI-II) for 'year by treatment' interaction effects.

Years	Treatments	PS (%)	IM (%)	TSW (g)	SG (%)	SDW (g)	SL (cm)	RL (cm)	VGI-I	VGI-II
2021	SP	98.00bc	2.00cd	4.34a	82.93c	0.031d	9.94a	2.41b	1024.00de	2.60d
2021	HP	98.42abc	1.58cde	4.26a	85.67bc	0.031d	10.18a	2.97a	1126.50bc	2.69d
2021	HBP	99.70a	0.30e	4.39a	88.33b	0.030d	10.28a	2.96a	1167.60ab	2.67d
2021	VPA	99.25abc	0.75cde	4.09ab	84.33c	0.029d	9.29bc	2.46b	990.30e	2.46d
2022	SP	91.69e	8.31a	3.76bc	78.63d	0.038b	9.80ab	2.53b	969.90e	2.97c
2022	HP	93.60d	6.40b	4.18a	82.25c	0.042a	9.87ab	3.06a	1064.20cd	3.43b
2022	HBP	99.45ab	0.56de	4.15a	93.75a	0.042a	9.94a	2.92a	1205.80a	3.89a
2022	VPA	97.73c	2.28c	3.55c	85.63bc	0.035c	8.73c	2.59b	968.50e	2.98c
LSD (0.05)		**1.70****	**1.70****	**0.36ns**	**3.62****	**0.002****	**0.60ns**	**0.32ns**	**68.32ns**	**0.23****
CV		**1.19**	**41.70**	**6**	**2.89**	**3.07**	**4.21**	**7.96**	**4.36**	**5.28**

Note: PS= Pure seeds, IM= Inert matter, TSW= Thousand seed weight, SG= Standard germination, SDW= Seedlings dry weight, SL= Shoot length, RL= Root length, VGI-I= Vigour index one, VGI-II= Vigour index two, SP=Self-pollinated, HP=Human pollinated, HBP=Honeybee pollinated, VPA= various pollinated agents.
Means within a column followed by the same letter are not significantly different from each other at 5% Probability level based on LSD test.

Conclusion and Recommendations

Methods of pollinations and cropping seasons had varied effects on onion quality seeds production and seed yields. The current study was to determine the effects of various pollination methods on onion seed yields and quality attributes. Pollination of onion by honeybee was found more effective than other pollination methods in terms of seed yields and quality attributes. In the onion seed production, seed yields and quality differ with the variation in pollination methods. Seeds produced by the combination of honeybee pollination method in insect proof cages were the most suitable for quality seed production of onion in the central part of Ethiopia. Onion seeds produced by honeybee pollination method gave the highest seed yields (910.42 kg/ha) followed by onion seeds yields (727.60 kg/ha) produced by various pollinating agents in the open field plots. The findings of this study suggested that pollination of onion seeds by honeybee is good for production of high seed yields, high physical purity and physiological seed quality. The results advised that seed producers should be aware of the effect of pollinating agents, their efficiency and well understand genetic seed quality relating with time and spatial isolations to obtain high seed yields and quality seeds.

However, further study is required on genetic purity of onions seeds either through grow out tests in the field condition or DNA finger printing in the laboratory to determine the spatial isolation of two or more onion varieties having similar or different seed classes produced in open fields and insect proof cages. Moreover, determination of isolation by time for two or more onion varieties is very useful. The current spatial isolation of two or more onion varieties that have similar or different seed classes which is set by Ethiopian standard authority was 1000 m for breeder and pre basic seeds which is very challenging in areas where shortage of land is critical.

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References

Abdul Baki, AA., and Anderson, D.J. 1973. Vigor Determination in Soybean Seed by Multiple Criteria. *Crop Sci.* **13:630-633.**

Ahmad, S., Muhammad, T., Karim, A., Jabbar, A., and Ahmad, G.h. 2003. Effect of Pollinators and Insecticides on Seed Setting of Onion (*Allium Cepa* L.). *Journal of Biological Science.* **3(3):305-308.**

Ahmed, H.I., and Abdalla, A.A. 1984. The role of honey bees as pollinators on onion (*Allium cepa* L.) seed production. *ISHS Acta Hortic.* **143:127-32.**

Aminpour, R., and Mortzavi, Bak, A. 2004. Effect of Planting Date and Intra-Row Spacing on Seed Yield and Germination of Onion (*Allium Cepa* L.) Cv.Texas Early Grano 502). *Pajouhesh & Sazandegi.* **62:64-69.**

Baswana, KS. 1984. Role of Insect Pollinators on Seed Production in Coriander and Fennel. *South Indian Hort.* **Pp:117-118.**

Brewer, J W. 1974. Pollination Requirements for Watermelon Seed Production. *J. Apic. Res.* **13(3):207-212.**

Chandel, R.S., Thakur, R.K., Bhardwaj, N.R., and Pathania, N. 2004. Onion Seed Crop Pollination: A Missing Dimension in Mountain Horticulture. *Acta Hortic.* **6(31):79-86.**

CSA. 2021. The Federal Democratic Republic of Ethiopia Report on Area and Production of Major Crops. Vol. I.

Deodikar, G.B., and Suryanaryana, M.C. 1972. Crop Yields and Bee Pollination. *Indian Bee J.* **4:53-64.**

ESA. 2016. Ethiopian Seed Standard Specification for Open Pollinated Onion Crops, Ethiopian

Statndards Authority (ESA), Addis Ababa, Ethiopia.
FAO. 2002. Production Year book. 56th ed. Rome, Italy.
Fiala F. 1987. Seed Vigour Test Methods. In Handbook of Vigour Test Methods. Zuerich, Swzerland: ISTA.
Hamilton, R.P. 1946. Onions need bees. *Gleanings Bee Cult.* **74: 23.**
Hussein Sadeghi, Adel, Mortaza Rasekh, and Namaghi. 2013. Effects of Insect Pollinators on Onion Seed Production Quality and Quantity. *Journal of Crop Protection.* **2(4):395-401.**
ISTA. 2016. International Rules for Seed Testing. International Rules for Seed Testing Association, Zurich, Switzerland. **12:345**.
Jaboñski, B., Skowronek, J., Woyke, H.W., and Doruchowski, R. W. 1982. Biology of Viticulture, Nectarization, Pollination and Fruiting of Male-Sterile Onion Lines (*Allim Cepa* L.). *Bee.Zesz.Nauk.* **26:57-104.**
Kalmath, B.S., and Sattigi, H.N. 2004. Effect of Bee Pollination by Using Attractants on the Qualitative Parameters of Onion Seed. *J. Pest Manag.Econ. Zool.* **12(1):97-99.**
Khokhar, K.M. 2009. Effect of Set-Size and Storage Temperature on the Incidence of Bolting, Bulbing, and Seed Yield in Two Onion Cultivars. *Scientia Horticulturae*, **122:187-194**. http://dx.doi.org/10.1016/j.scienta.2009.05.008.
Kumar, J., Mishra, R.C., and Gupta, J.K. 1989. Effect of Honeybee Pollination on Onion (Allium Cepa L) Seed Production. *Indian Bee J.* **51(1):3-5.**
Mahesh, C.G. 2007. Effect of Seed Source, Growing Season and Genotypes on Seed Yield and Quality in Groundnut (Arachis Hypogaea L.). *Agricultural sciences, Dharwad.*
Meer, Q. P., Van der, and J. L., Van Bennekom. 1968. Research on Pollen Distribution in Onion Seed Fields. *Euphytica.* **17(2):216-219.**
Muller, Hermann. 1883. The Fertilization of Flowers. London: *Macmillan.*
Prasad, R., Chand, H., and Singh, R. 2000. Effect of Boney Bee, Apis Mellifera Pollination on the Yield and Germination of Onion (*Allium Cepa* L) seeds. *Shashpa.* **7(1):53-55.**
Rao, G. M., and Lazar, M. 1983. Studies on Bee Behavior and Pollination in Onion (*Allium Cepa* L.). Proceedings of the 2nd International Conference on Apiculture in Tropical Climates. *New Delhi.* **3:580–589.**
Ruszkowaski, A., and Bilinski, M. 1984. An Attempt to Use Bumblebees (Bombus Latr.) and Alfalfa Moth (Megachile Rotundata F.) for Pollination of Cucumber Breeding Material. *Pszczelnicze Zeszyty Naukowe (Poland).* **28:175-184.**
Saeed, A., Muhammad, T., Karim, A., Jabbar, A., and Ahmad, G. 2008. Effect of Pollinators and Insecticides on Seed Setting of Onion (Allium Cepa L.). *Ann. Appl. Boi.* **130(3):497-506.**
Sajjad, Asif, Shafqat Saeed, and Asad Masood. 2008. Pollinator Community of Onion (*Allium Cepa* L.) and Its Role in Crop Reproductive Success. *Pakistan Journal of Zoology.* **40(6):451-456.**
Sanduleac, E. 1961. The pollination of vegetable seed plants. *Apiculture.* **14: 25-26.**
Singh, J.P., and Dharamwal, S.C. 1970. The Role of Honey Bee in Seed Setting of Onion. *Indian Bee J.* **32(1/2):23-26.**
Tolon, B. and Duman, Y. 2003. The Effect of Pollination by Honeybee (Apis Mellifera) on Onion (*Allim Cepa* L.) Seed Production and Quality. *In XXXVIII Apimondia Ineternational Apicultural Congress.* **554.**
Van der Meer, Q.P. and Van Bennekom, J.L. 1968. Research in pollen distribution in onion seed fields. *Euphytica.* **7:216-19.**
Woyke, H.W. 1981. Some Aspects of the Role of the Honeybee in Onion Seed Production in Poland. *Acta Hortic. J.* **111: 91-98.**
Yucel, B., and Duman, I. 2005. Effect of Foraging Activity of Honey Bee (*Apis Mellifera* L.) on Onion (*Allium Cepa* L.) Seed Production and Quality. *Pakistan J. Biol. Sci.* **8(1):123-126.**