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## Effect of pollination on flower abortion, fruit set and fruit production in four mangroves species

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### Abstract

The study was conducted in Nyeke and Michamvi mangrove forests in the southern region of Unguja Island in Zanzibar. This study was designed to examine the effect of pollination on buds, flowers, fruit sets and fruits of four mangrove species namely *Rhizophora mucronata*, *Bruguiera gymnorhiza*, *Ceriops tagal* and *Avicennia marina*. The selected mangrove branches were free from pest and diseases and have been selected randomly, and observations were carried out twice a week in the period of nine months. A total of 80 trees (20 trees per specie) were randomly selected. The height of the selected trees was approximately 3.5m. Four treatments were established on flowers of the selected branches. The treatments were: open pollination (control), open plus hand cross pollination (pollen supplement), closed self-pollination (bagged), and closed plus hand cross pollination (bagged supplement). The results showed that in all four mangrove species there were significant differences in the number of buds, flowers, fruit set, visitors, visits and fruits. High number of flowers aborted was reported in bagged treatments in both sites compared to other species. But the number of fruits produced was higher in bagged supplement treatments in both sites. The increases of the number of flowers produced also reflecting to the number of buds produced. Increase number of flowers concurrently increase on the number of fruit set in all species, although *A marina* showed a weak relationship. The study concluded that the increase on the number of mangroves fruits not only depends on the number of fruit set and other reproduction variables, but also there was other abiotic and biotic factors that require intensive investigation.

**Keywords:** Bud, flower, fruit, pollination, visitors, visits

### Introduction

Pollination by insects comprises a vital ecosystem service, as reproduction and yields of many flowering wild <sup>[1]</sup> and crop plants <sup>[2]</sup> benefit from faunal pollinators. Long-term declines in pollinator populations and related threats to plant reproduction have led to concerns of a widespread loss of pollination services in which pollen-limited plants will suffer reduced yields due to declining pollen supply <sup>[3-6]</sup>.

Mangroves are pollinated by a diverse group of animals including bats, birds, and insects <sup>[7]</sup>. Pollen is deposited on the animals as they deeply probe the flowers for nectar and subsequently they transfer the pollen to the stigma of another flower <sup>[8]</sup>. The identity of pollinators differs among species. For example, *Lumnitzera littorea* is pollinated primarily by birds while *L. racemosa* and the small-flowered *Bruguiera gymnorhiza* are pollinated by insects <sup>[9]</sup>. Sunbirds visit and may pollinate *Acanthus ilicifolius* <sup>[10]</sup> and the large-flowered *Bruguiera hainesii* <sup>[11, 12]</sup>. Differences in flower visit duration among pollinators have been implicated in influencing pollinator effectiveness. Visit duration has been shown to be positively related to pollinators' effectiveness <sup>[13-15]</sup>. Pollinator competition was also inferred from a study by Landry <sup>[14]</sup> who found that the relationship between number of flowers, fruits set, insect visitation, and visitation rates *L. racemosa* increased significantly when *A. germinans* stopped flowering. Fruit size and weight was observed to be affected by lack of pollen grain, whereby less seed production were recorded for wind-pollinated fruit and the highest for hand-pollinated fruit of apple <sup>[16]</sup>.

Over 80% of the total pollination activities are performed by insects, and bees contribute nearly 80% of the total insect pollination, and therefore, they are considered the best pollinators <sup>[17]</sup>. Another value of pollination lies in its effect on quality and efficiency of crop production. Inadequate pollination can result not only in reduced yields but also in delayed yield and a high percentage of culls or inferior fruits <sup>[18]</sup>.

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Despite this huge economic contribution, pollinators especially insects are declining globally due to disruption of interacting factors [19]. It has been reported that over the last 25 years, there has been a significant decline in the diversity of pollinators globally but in particular of butterflies and bumblebees [20].

Although it is generally accepted that pollinators are important in fruit formation it is important to understand exactly how this happens. The effectiveness of pollinators can be determined by the number of fruits set and fruit produced. There is no information on the role of pollinator in fruit set and fruit production in mangroves of Zanzibar. Therefore, this study investigated the effect of pollination on flowers and fruit set in four species of tropical mangroves in Zanzibar. These findings will provide baseline information for further research and make desirable additional information on the biology of pollination in mangroves ecosystem in East Africa and globally.

## Materials and Methods

### Field experiments

Mangrove trees of *Rhizophora mucronata*, *Bruguiera gymnorhiza*, *Ceriops tagal* and *Avicennia marina* were selected randomly in Nyeke and Michamvi forests. Precaution was taken to ensure that all selected tree branches were of about the same size. Plants with dry branches or those which showed symptoms of diseases or pest attack were excluded. The height of selected trees was approximately 3.5m. In of the two sites 80 trees (20 trees per specie) were randomly selected and on each tree four reproductive branches were selected and tagged. Mature flowers free from diseases, pest and malformation were selected for use in this experiment. Four treatments were established on flowers of the selected branches. The treatments were: open pollination (control), open plus hand cross pollination (pollen supplement), closed self-pollination (bagged), and closed plus hand cross pollination (bagged supplement). The experiment was carried out during peak period of flowering for each of the mangrove species. Sterile camel brush was used to transfer mature sticky pollen grains to the stigma. Hand cross pollination was carried out between flowers (pollen) from the same tree mixed with flowers of separate trees without emasculation. In all treatments an odorless jelly or grease was applied to the tagged branches to prevent and deter ants, spiders, snakes and crustaceans from disturbing the experiments.

Bagged and open treatments were regularly (checked twice a week) and the number of flowers formed, as well as the number of flowers aborted were recorded. The time for monitoring was dependent on low and high tides of sea. Generally, observation for this experiment was done in the morning, from 6.00 am to 10.00 am, because most flowers were found open at this time. Observations were carried out throughout peak periods of six months from September 2013 to February 2014 depending on mangroves species. After the flowering period, the number of fruits set was monitored. The number of fruits aborted was also recorded. The extent of flower and fruit abortion was determined by observing and recording wilting and subsequent falling of flowers and fruits. For *Rhizophora mucronata*, *Bruguiera gymnorhiza*, *Ceriops tagal* observations period was six months and *Avicennia marina* was 5 months. The data collection sheet included: date, sites, mangroves species, treatments, percentage number of buds, number of flowers, number of flowers aborted, number of fruits aborted and number of fruits set:

1. Open pollination (control): open natural pollination was conducted by leaving inflorescences open for free access by all vertebrate pollinators, invertebrate pollinators, and

self- and wind pollination. In each treatment, the selected branches were tagged when still in their bud stage.

2. Open and hand cross pollination (pollen supplement): In this treatment the inflorescences were left open for free access by pollinators. Thus pollination included self, cross (autogamous and geitonogamy) and wind pollination. In addition, pollen grain from three trees at least 5 m away (cross fertilization/allogamy), was used to supplement pollination, by brushing anthers gently across stigmas of experimental flowers during peak flowering period. The selected branches were tagged when the flowers were still in their bud stage.
3. Closed self-pollination (bagged): In this treatment selected branches were enclosed in bags of fine nylon mesh gauze (10  $\mu$ m) to exclude pollination by insects, bats, and birds with little wind influence. The selected branches were tagged when the flowers were still in their bud stage.
4. Hand closed cross pollination (bagged supplement): The selected reproductive branches of mangroves were enclosed in a plastic bag with mesh pores measuring 10  $\mu$ m sizes in order to prevent small insects, birds and bats from entering, and limiting penetration of wind borne pollen. This experiment was conducted to investigate how cross pollination could be used to supplement self-fertilization. Manual cross pollination was done by collecting pollen from donor flowers with fine sterilized forceps and rubbing the pollen grains across the stigmas of receiving flowers using camel brushes.

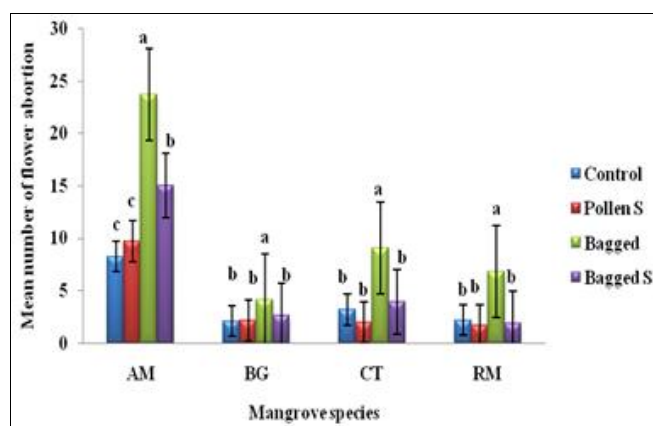
### Statistical analysis

For all statistical analysis Proc GLM SAS version 9.3 (SAS Institute 2016, Cary, NC) was used. ANOVA test was used to compare different treatments, species and sites. Significant variation were compared at 95% ( $\alpha=0.05$ ). Post-hoc test SNK (Student Newman Keuls) ( $p < 0.05$ ) was to separate the means.

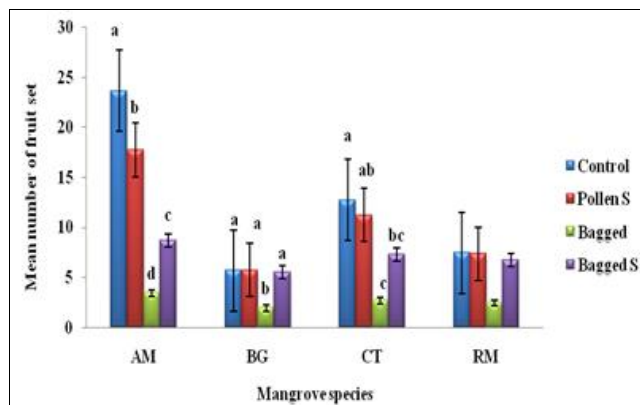
## Results

### Flower abortion

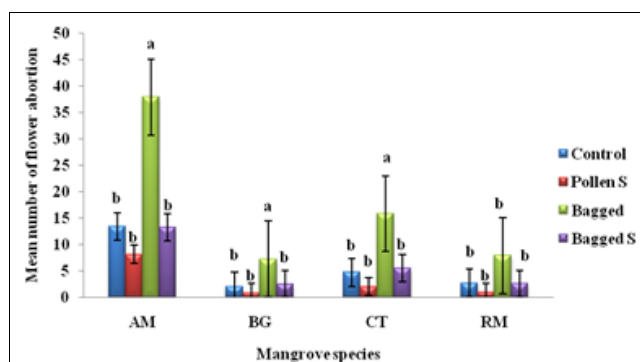
Results showed that the mean number of aborted flowers differed significantly among the four mangroves species and treatments (d.f = 15,  $F=10.45$ ,  $P < 0.0001$ ) (Fig. 1 and 2). Results of effects of treatment on flower abortion are shown in Figure 1. Regardless of site or species, bagged (without pollen supplement) experiment had the highest flower abortion.



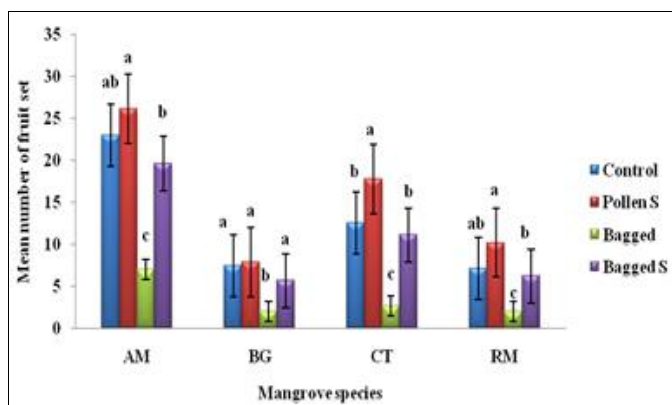
**Fig 1:** Mean number ( $\pm$  SE) of aborted flower in Nyeke forest. Means with different letters within a species are significant different ( $P < 0.05$ ) (n= 196).



**Fig 2:** Mean number ( $\pm$  SE.) of fruits set in Nyeke forest. Means with different letters within a species are significant different ( $P < 0.05$ ) (n= 196).



**Fig 3:** Mean number ( $\pm$  SE.) of aborted flower in Michamvi forest. Means with different letters within a species are significant different ( $P < 0.05$ ) (n= 196).



**Fig 4:** Mean number ( $\pm$  SE.) of fruit set in Michamvi forest. Means with different letters within a species are significant different ( $P < 0.05$ ) (n= 196)

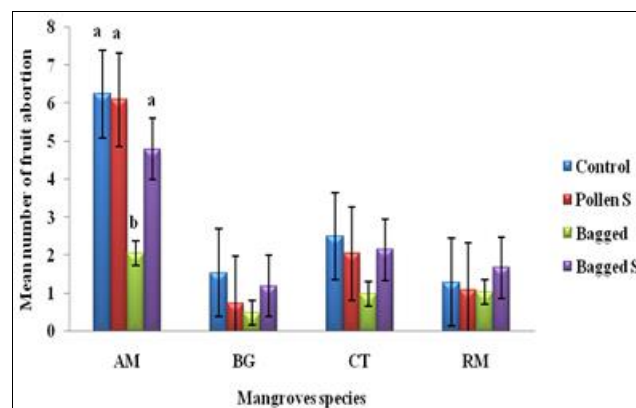
**Fruit set**

The results show that the number of fruits set differed significantly among mangrove species and treatments (d.f = 15,  $F=9.48$ ,  $P < 0.0001$ ) (Fig. 4). Generally fruit set in Nyeke was the highest in the control experiment followed by open

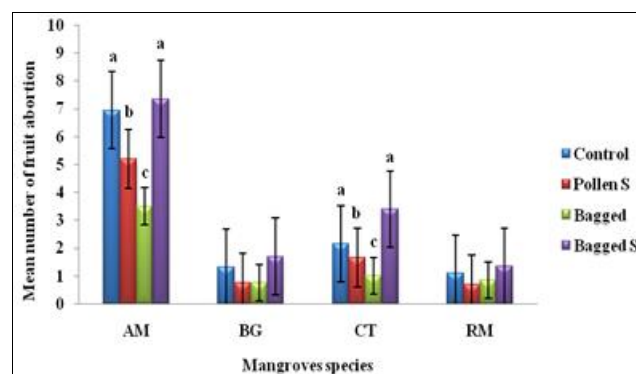
pollination with supplement, bagged with supplement and bagged treatments respectively (Fig. 4). In Michamvi forest, fruit set was highest in open pollination with supplement (Fig. 4).

**Fruit abortion**

There were significant differences in the mean number of fruits aborted among mangroves species and treatments (d.f = 15,  $F= 5.85$ ,  $P < 0.0001$ ) (Fig 3). The mean numbers of fruit abortion in Nyeke did not differ among treatments except in *A. marina* where it was significantly less in bagged treatment (Fig. 5). On the other hand open pollinated flowers with pollen supplement had the highest fruit abortion in Michamvi forest (Fig. 6).



**Fig 5:** Mean number ( $\pm$  SE.) of fruits aborted in Nyeke forest. Means with different letters within a species are significant different ( $P < 0.05$ ) (n= 196).



**Fig 6:** Mean number ( $\pm$ SE.) of fruits aborted in Michamvi forest. Means with different letters within a species are significant different ( $P < 0.05$ ) (n= 196).

**Fruit production**

In Nyeke forest the highest number of fruit set was recorded in control and pollen supplement treatments (Table 1, Fig. 7). The least number of fruit set was recorded in the bagged treatment. In Michamvi forest the highest fruit production was in the control and pollen supplement treatments (Table 1, Fig. 8).

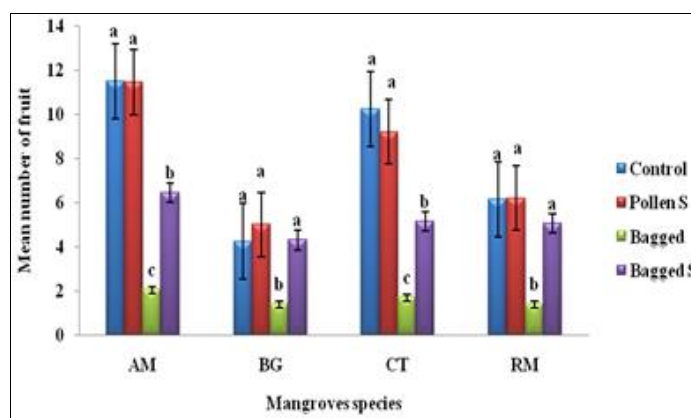
**Table 1:** Percentages of flowers aborted, fruits set, fruits abort and fruits produced by site

Mangroves species	Treatments	Forest sites							
		Nyeke mangrove forest				Michamvi mangrove forest			
		Flowers aborted (%)	Fruits set (%)	Fruits aborted (%)	Fruits Produced (%)	Flower aborted (%)	Fruits set (%)	Fruits aborted (%)	Fruit produced (%)
<i>Avicennia marina</i>	Control	20	63	19	28	35	56	17	41
	Pollen S	28	60	17	33	31	67	13	53
	Bagged	64	9	6	6	78	14	7	7

	Bagged S	45	26	14	19	37	55	20	34
<i>Bruguiera gymnorrhiza</i>	Control	28	62	17	46	21	76	13	62
	Pollen S	23	76	10	66	10	88	8	80
	Bagged	56	28	7	10	75	20	8	10
	Bagged S	30	64	14	50	30	67	23	47
<i>Ceriops tagal</i>	Control	18	76	17	62	27	71	12	57
	Pollen S	13	73	14	59	10	89	8	77
	Bagged	71	25	8	6	83	14	5	8
	Bagged S	32	59	14	42	32	65	18	45
<i>Rhizophora mucronata</i>	Control	22	75	13	62	26	69	11	57
	Pollen S	18	77	12	65	9	89	6	84
	Bagged	66	24	10	4	76	15	9	6
	Bagged S	21	74	18	55	29	69	15	54

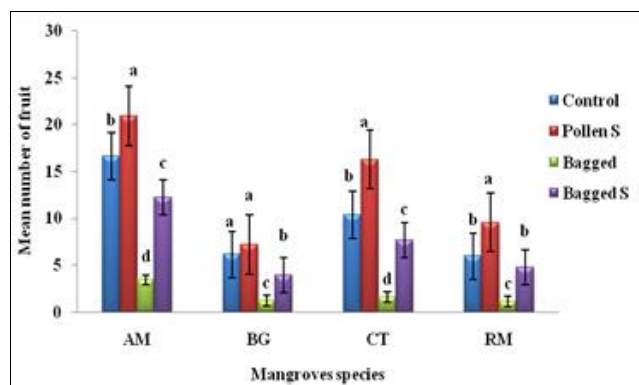
**Table 2:** Summary of interaction between flower abortions, fruit set, fruit abortion and fruit production for four mangrove species and sites.

Mangrove species	Interaction effects		The mean values		
	Sites and Treatments	flower abortion	fruit set	fruit abortion	fruit
<i>A. marina</i>	Michamvi Bagged	37.95a (29)	6.95cd (5)	3.50cd (8)	3.45e (4)
	Michamvi Bagged S	13.30c (10)	19.60b(15)	7.35a (17)	12.25c(14)
	Michamvi Control	13.45c (10)	22.95ab(18)	6.95a (16)	16.60b(20)
	Michamvi Pollen S	8.15d (6)	26.15a(20)	5.20bc(12)	20.90a(25)
	Nyeke Bagged	23.70b (18)	3.45d (3)	2.05d (5)	2.05e (2)
	Nyeke Bagged S	15.00c (12)	8.70c(7)	4.80bc(11)	6.45d (8)
	Nyeke Control	8.25d (6)	23.65ab(18)	6.25ab(15)	11.50c(14)
	Nyeke Pollen S	9.75dc (8)	17.75b (14)	6.10ab(14)	11.45c(14)
<i>B. gymnorrhiza</i>	Michamvi Bagged	7.25a (31)	1.95b (5)	0.75 (9)	1.25c (4)
	Michamvi Bagged S	2.55b (11)	5.65ab (14)	1.70 (20)	3.95b (12)
	Michamvi Control	2.10b (9)	7.45a (18)	1.30 (15)	6.15ab(18)
	Michamvi Pollen S	0.90b (4)	7.90a (19)	0.75 (9)	7.20a (21)
	Nyeke Bagged	4.10ab (17)	1.94b (5)	0.50 (6)	1.40c (4)
	Nyeke Bagged S	2.60b (11)	5.50ab (13)	1.20 (14)	4.30b (13)
	Nyeke Control	2.10b (9)	5.70ab (14)	1.55 (18)	4.25b (13)
	Nyeke Pollen S	2.15b (9)	5.75ab (14)	0.75 (9)	5.00ab(15)
<i>C. tagal</i>	Michamvi Bagged	15.85a (34)	2.60d (3)	1.00b (6)	1.60e (3)
	Michamvi Bagged S	5.55bc (12)	11.10bc(14)	3.40a (21)	7.70c (12)
	Michamvi Control	4.75bc (10)	12.55b (16)	2.15ab(14)	10.40b(17)
	Michamvi Pollen S	2.05c (4)	17.75a (23)	1.65 b (10)	16.26a(26)
	Nyeke Bagged	9.10b (20)	2.70d (3)	1.00b (6)	1.70e (3)
	Nyeke Bagged S	3.95c (9)	7.30c (9)	2.15ab(14)	5.15d (8)
	Nyeke Control	3.20c (7)	12.75b (16)	2.50ab(16)	10.25b(16)
	Nyeke Pollen S	2.00c (4)	11.25b (14)	2.05a (13)	9.20bc(15)
<i>R. mucronata</i>	Michamvi Bagged	7.90a (29)	1.95b (4)	0.85 (9)	1.15c (3)
	Michamvi Bagged S	2.60bc (10)	6.20ab (13)	1.35 (15)	4.80b (12)
	Michamvi Control	2.70bc (10)	7.10a (14)	1.10 (12)	5.95b (15)
	Michamvi Pollen S	1.00c (4)	10.20a (21)	0.70 (8)	9.55a (24)
	Nyeke Bagged	6.80ab (25)	2.45b (5)	1.05 (12)	1.40c (3)
	Nyeke Bagged S	1.89c (7)	6.73ab (14)	1.68 (18)	5.05b (13)
	Nyeke Control	2.20bc (8)	7.45a (15)	1.30 (14)	6.15b (15)
	Nyeke Pollen S	1.70c (6)	7.35a (15)	1.10 (12)	6.20b (15)
	SE	1.72	1.61	0.64	0.91
	P Values	<.0001	<.0001	<.0001	<.0001



**Fig 7:** The mean ( $\pm$ SE) number of fruits produced in Nyeke forest. Means letters within a species are significant different ( $P \leq 0.05$ ) ( $n = 196$ ).





**Fig 8:** The mean ( $\pm$ SE) number of fruits produced in Michamvi forest. Means with different letters within a species are significant different ( $P \leq 0.05$ ) ( $n = 196$ ).

### Interaction between flower abortions, fruit set, fruit abortion and fruit production of four mangrove species and sites.

Summary of interaction of flower abortion, fruit set, fruit abortion and fruit production is shown in Table 2. There were variation on interaction between flower abortion, fruit set, fruit abortion and fruit on four mangrove species and sites ( $P < 0.0001$ ) (Table 2). The treatments showed that the mean number of aborted flowers were significant different. In *A. marina* it was observed that high number of flowers aborted in bagged treatment in both sites compared to other species. Michamvi recorded the highest abortion of 29% than Nyeke of 18%. The least number of flower abortions were recorded 6% in pollen supplement in Michamvi and control treatment in Nyeke. In contrast, in *B. gymnorrhiza*, the bagged treatment recorded highest abortions and are significantly different from other species. Likewise, high number of fruit abortion in *C. tagal* were found in bagged in Michamvi (34%) and was significantly different from other species. Correspondingly, the *R. mucronata* in bagged experiment have shown to produce high number of fruit abortion, while pollen supplement was observed the least compare to the other species the four mangrove species, bagged experiment showed a high percentage of flower and fruit abortion and lowest number of fruits produced compared to other treatments. These results are similar to those obtained by Tomlinson *et al.* [21] who reported a threefold increase in fruit set in open cross hand pollination treatment in *Rhizophora mangle* compared to self-pollinated treatment. The lower percentages of fruits set and fruit produced could be attributed to the exclusion of pollinators, resulting in decrease in the number of fertilized flowers and the subsequent reduced fruit emergence. These results agree with those of [20 Also, fruit set in bagged treatment of *A. marina* in south eastern Australia reported similar results; produced few numbers of fruits than unbagged or control [22, 23].

In Michamvi forest fruit set was the highest in the open plus supplement treatments while in Nyeke it was highest in the control. This indicates that there could be pollinator limitation in Michamvi. Nyeke is close to Jozani conservation area and near cultivated farms and both could be the source of pollinators. Michamvi, on the other hand, is more than 20 km from Jozani thus explaining why pollen supplement boosted pollination and the subsequent fruit production. This further shows that plants can compensate fertilization through hand cross pollination. In addition bagged experiments had high rates of flowers abortion compared to the control and pollen supplemented treatments. This could mean that effects of lack

of pollination start much earlier before fruit set. Thus the cause of reduced number of fruits is a combination of flower and fruit abortion.

It is also possible that plant ability to supply adequate resources such as nutrients to sustain fruit growth plays a role in fruit abortion. Elsewhere in this study it was found that there was young and mature fruits in CT, RM and BG throughout the year. This is likely to affect the number of fruits set and may be indirectly [24] that, the existence of emerging and mature fruits can inhibit subsequent fruit set and growth of young fruits. This inhibition could be triggered by antagonism for available assimilates, by dominance due to production of plant growth regulators from the developing fruit [25, 26, 27]. Thus this study has established that cross pollination is an important factor in fruit production and subsequent regeneration of mangroves.

### Conclusions

For all four mangroves species the number of flowers produced depends on the number of buds produced. It is not very clear whether fruits set and fruits produced depend on the number of pollinators and visits. However the study concludes that there is mutual relationship between number of fruits produced and pollinators. The presence of fruits in the bagged treatments indicates that the four mangroves species not only depend upon insects and other pollinators for pollination but also are self-pollinated. On the other hand the study conclude that abiotic and biotic factors that require intensive investigation on mangroves pollination and fruits production.

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