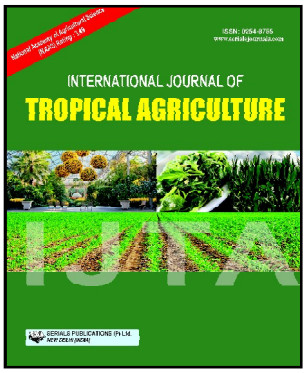

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Influence of Pollen viability on Quantity and Quality of Seeds in Bitter Gourd (*Momordica charantia* L.)

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Abstract: Studies on pollen grains of Bitter gourd (Momordica charantia L.),which belongs to the family Cucurbitaceae, was carried out in commercial vegetable farm in Kerala, India. Observations were done at different time intervals in the flowering season to understand influence of pollen viability on quantity and quality of seeds. Germination assays showed highest percentage of viable pollen grains in the initial phase of the day and middle phase of the flowering season and lowest in late phase of day and late phase of flowering season.

***Key words*:** Bitter gourd, Pollen, viability, Seed production

INTRODUCTION

Pollen viability is an important factor for plant genetic variability, especially for those plants in which cross fertilization prevails over self-fertilization as it reveals the male reproductive capacity and enables different allele combinations (Karasawa, 2009; Divakara *et al.* 2010). The issue of pollen viability has received attention as there is great variability among species in the effective life spans of pollen grains (Stanley and Linskens,1974; Shivanna and Johri,1985). According to Dantas *et al.* (2005) and Tuinstra and Wedel (2000), assessment of pollen fertility is a preliminary and indispensable condition for genetic crop breeding.

MATERIALS AND METHODS

The crop selected for this study *Bitter gourd (Momordica charantia* L.*)* is considered to be native of India. It is a monoecious annual plant of slender climbing habit. It is also grown as an ornamental and is used extensively in folk medicine (Heiser, 1979). Flowering behavior varies with cultivar, climatic conditions, and cultural practices (Deshpande *et al.* 1979). The yellow flowers are solitary in the leaf axils. corolla with five

petals. In staminate flowers the stamens are free, filaments are free, and anthers are united. The pistillate flower of bitter gourd consists of an inferior ovary and a three-lobed, wet stigma that is attached to a columnar, hollow style (Pillai, *et al.* 1978). Long photoperiods cause staminate flowers to bloom up to 2 weeks before the pistillate flowers while short days have the reverse effect (Huyskens, *et al.* 1992). The fruits are long and oval, narrowed or tapering toward both ends. It is covered with blunt tubercles. They are green when unripe, turning to an orange yellow colour when ripe.

The study on the selected crop was conducted in the field at Madayi which is located between 12°1'N and 75°15'E in Kerala, India. A randomized complete block design with 6 replicates of 2 beds was made for this. There were 2 beds /replicate and 12 hills /bed. All plants were grown on raised bed of 2m. wide and 6m. length. The field was directly seeded with 3-5 seeds /hill. Upon emergence (germination) the plants were thinned to one /hill. Spacing between beds was 1.5m. with interplant spacing of 1m. and the inter-replicate spacing of 10m. Each replicate measured 33sq.m.with sequential plantings.

To quantify pollen grains and the influence of its viability on seed set observations were made on randomly selected plants. One plant from each bed was selected for observation. Thus 12 staminate and 12 pistillate flowers were selected for study, i.e. 4 staminate and 4 pistillate flowers each during each diurnal phase. Observations were carried out in three diurnal phases - initial phase (idp), middle phase (mdp) and late phase (ldp) according to the longevity of flowers and peak time of pollinator visitation [(idp) 0600 h.-0800 h., mdp: 0800-1000 h., lsp: 1000-1200 h.]. Duration of each phase was two hours. They were made for 12 days during initial phase (ISP), 18 days during middle phase (MSP) and 12 days during late phase (LSP) of flowering season. Flowers at the time of anthesis and at the end of each phase of pollination were removed and pollen grains were collected by using a brush. Pollen grains from each sample were washed with water to a petridish for further counting with a counting chamber. Viability of pollen grains were tested using Brewbaker and Kwack's culture medium (Brewbaker and Kwack, 1963). Sample of pollen grains were dipped in 30 ml of the medium and kept it for 2-3 h. in a petridish. Viability was measured based on the number of pollen grains having pollen tube growth.

The seed set was determined from sub samples of fruits. The fruits were thawed, cut longitudinally and partially along the convex side. Seeds were manually extracted from the fruits. Along with the developed seeds (ovules) unfertilized ovules were counted. Unfertilized ovules were taken as nonviable seeds. To confirm viability the seeds were soaked in water and kept for 7-16 days to germinate and the number of sprouted and non sprouted seeds were noted.

All observations were made on warm sunny days. The data from each diurnal phase and seasonal phase were pooled for analysis. Statistica '99 version was used to carry out all statistical analysis.

RESULTS

Pollen Count

The mean number of pollen grains produced by staminate flowers were different in different phases of the season (Fig.1). It was highest in middle phase (MSP) (16861.08±55.86) than in initial phase (ISP) (7899.58±15.82) and late phase (LSP) (6703.66±14.27) of the season. At anthesis staminate flowers contained maximum pollen grains /flower and the number of pollen grains remaining on anthers decreased over time of day ISP [idp (7899.58±15.82); mdp (6186.33±4.47); ldp (3727.33±13.43)]; MSP [idp (16861.08±55.86); mdp (12526±4.39); ldp (3338.33±68.55)] LSP [idp (6703.66±14.27); mdp (5518.5±3.61); ldp

(3876.25±12.98). The mean number of pollen grains produced was highest in the initial diurnal phase (16861.08±55.86) of middle seasonal phase and lowest in the late diurnal phase (3876.25±12.98) of late seasonal phase. The counts were significantly different in diurnal phases of each seasonal phase such as ISP ($p<0.05$); MSP ($p<0.05$) and LSP ($p<0.05$). Mean pollen count between the seasonal phases were also significantly different ($p<0.05$)

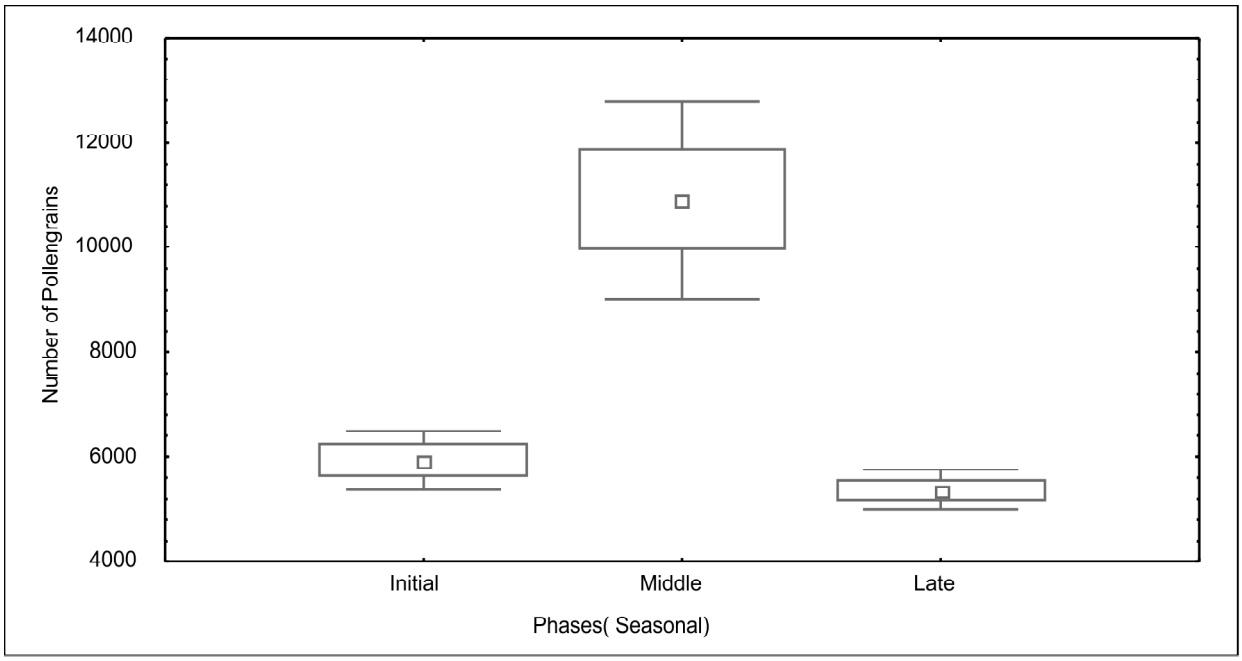

Figure 1: Mean pollen counts in different phases of the season

Pollen Viability

When viability of the all pollen grains produced was tested, great variation in the number of viable pollen grains was observed. Viability of pollen grains was found to decrease through diurnal phases of each season, ISP [idp (965.5±13.10); mdp (883.75±10.06); ldp (816.33±7.32)]; MSP [idp (4062.5±21.67); mdp (3761.66±22.18); ldp (3208.83±12.97)]; LSP [idp (748.91±9.48); mdp (684.08±9.83); ldp (550.91±10.31) and the variations in diurnal phases of each season were significantly different [ISP ($p<0.05$); MSP ($p<0.05$); LSP ($p<0.05$)]. Viability increased from initial phase of the season to middle phase (Fig.2). Most viable pollen grains were observed in initial diurnal phase of middle phase of the season (4062.5±21.67). In late diurnal phase of late seasonal phase least number of viable pollen grains were found (550.91±10.31).Viability of pollen grains between the seasonal phases were also significantly different ($p<0.05$).

Number of Seeds

Mean number of seeds increased from initial phase of the season ISP [idp=2.00±1.47; mdp=5.16±0.93; ldp=0.41±0.51] to middle phase of the season MSP [idp=6.91±3.31; mdp=20.75±2.30; ldp=0.83±0.93] and then decreased to late phase LSP [idp=1.16±1.0; mdp=2.25±1.35; ldp=0.33±0.49] of the season (Fig.3). As non pollinated flowers were aborted no seeds were recorded. Highest number of seeds were recorded in middle diurnal phase (20.75±2.30) of middle seasonal phase. In late diurnal phase of late seasonal phase lowest number of seeds (0.33 ±0.49) were recorded. Mean number of seeds within each

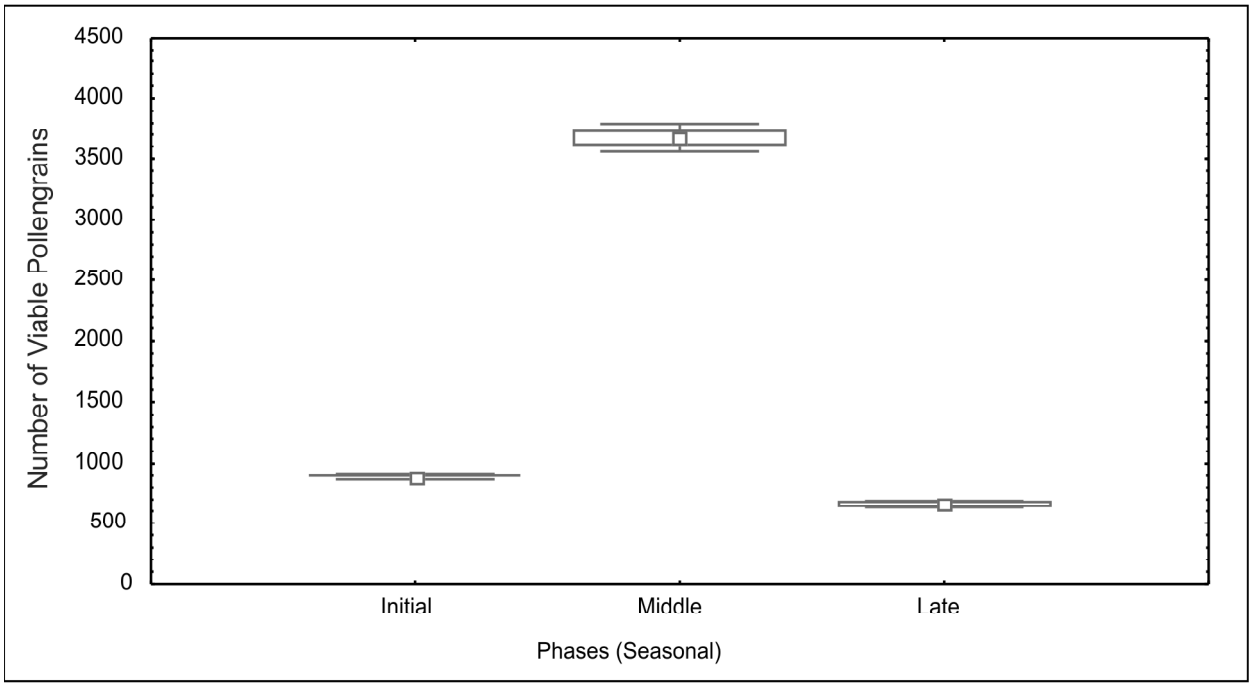

Figure 2: Mean pollen viability in different phases of the season

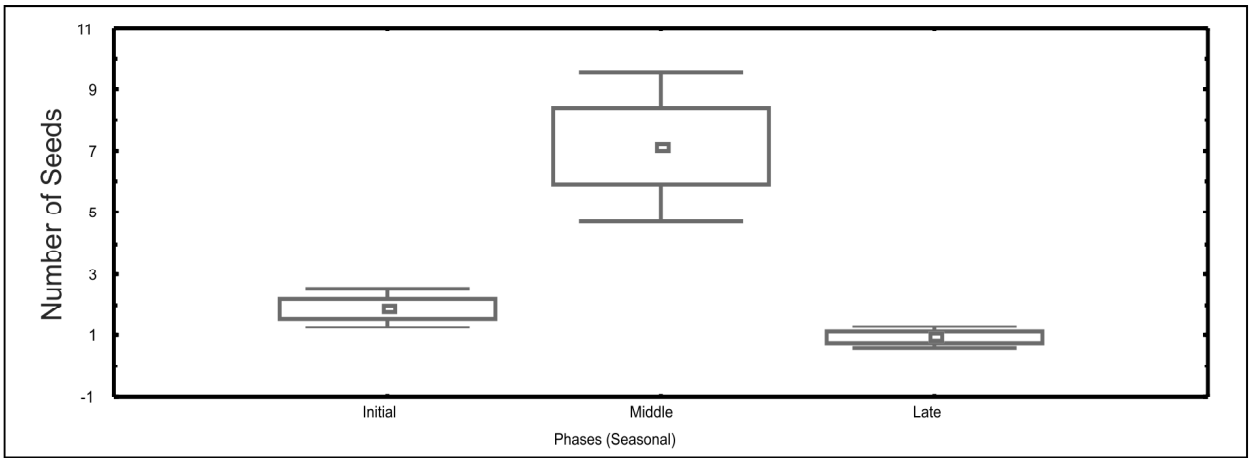

Figure 3: Mean number of seeds in different phases of season

seasonal phase such as ISP ($p<0.05$), MSP ($p<0.05$), LSP ($p<0.05$) and between the seasonal phases were significantly different ($p<0.05$).

Viability of Seeds

Mean number of viable seeds increased from initial phase of the season [ISP (idp=1.83±1.40; mdp=4.25±0.75; ldp=0.33±0.49)] to middle phase of the season [MSP (idp=6.08±2.91; mdp=19.33±1.96; ldp=0.75±0.86)] and then decreased to late phase [LSP (idp=0.58±0.51; mdp=1.33±0.88; ldp=0.08±0.28)] of the day and season (Fig. 4). Maximum viable seeds were recorded in middle diurnal phase (19.33±1.96) of middle seasonal phase. In late diurnal phase of late seasonal phase minimum number of viable seeds (0.08±0.28) were found. As non pollinated flowers were aborted no viable seeds were recorded. Number of viable

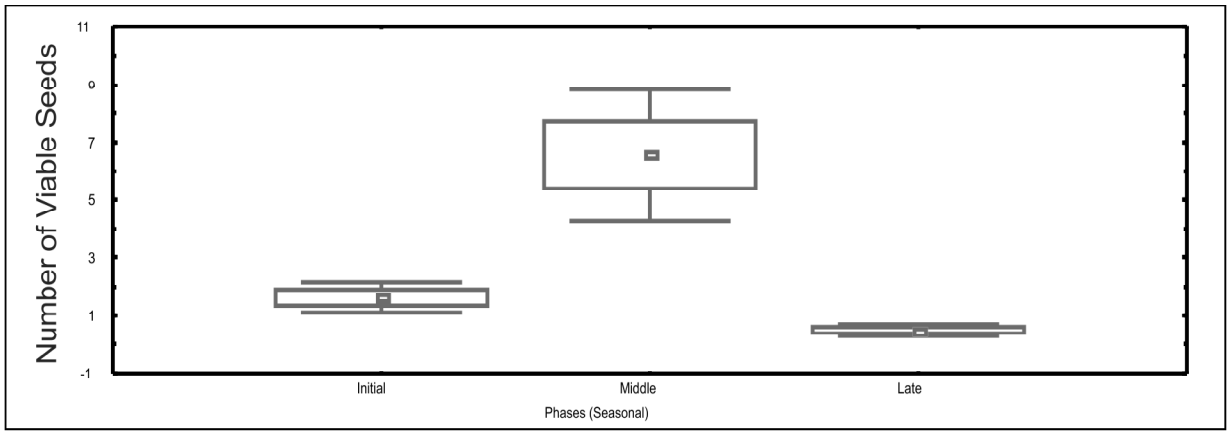

Figure 4: Mean number of viable seeds in different phases of season

seeds within each seasonal phase such as ISP ($p<0.05$), MSP ($p<0.05$), LSP ($p<0.05$) and between the seasonal phases were significantly different ($p<0.05$).

Correlation between Pollen viability and Seed Count

A positive correlation was found between number of viable pollen grains and seed count($r=0.64$) (Fig. 5).

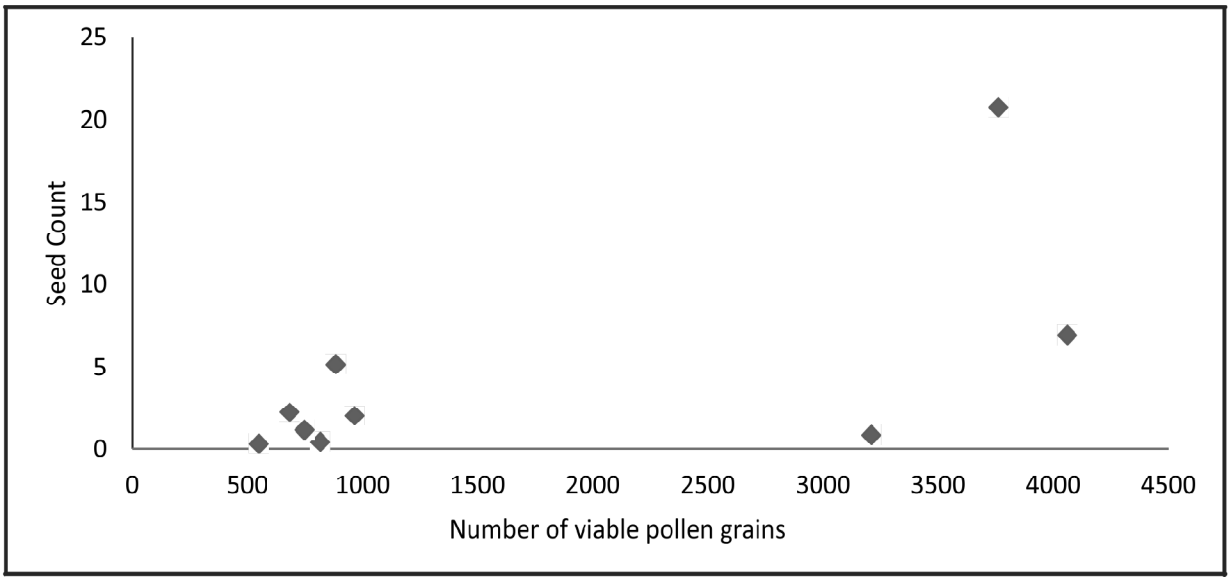

Figure 5: Correlation between pollen viability and seed count

DISCUSSION

In the present study it was observed that the quantity and quality of seeds were influenced by viability of pollen grains. Intraspecific variations in the pollen loads deposited on stigmas may influence both the number and quality of the eventual progeny (Herrera, 2002). Pollen count and pollen viability were found increasing from initial phase (ISP) to middle phase (MSP) and then decreasing to late phase (LSP) of the season. It may be due to the size variation of flowers produced. But both pollen count and pollen viability decreased over the day. Maximum number of pollen grains on anthers would be available during initial phase of day (idp) in all

seasonal phases and exponentially decrease thereafter due to pollen removal as the studies on pumpkin by Willis and Kevan (1995), on cucumber by Stanghellini *et al*. (2002) and Leena (2011).

The numbers of seeds were also found increasing from initial phase to middle phase and decreasing to late phase of the day and season. As insect visits increased there was a highly significant increase in the number of mature seeds that developed. Increased seed set in middle phase as a result of insect pollination could be due to greater number of viable pollen grains in the plots and greater number of ovules they contain. So efficiency of pollinators has so often determined the number of seeds the flowers set (Schemske and Horvitz, 1984; Motten, 1986; Young, 1988). Reduction in seed set in late phase was due to reduction in pollen deposition compared to middle phase and reduction in insect number. The threshold effects, nonlinear dose-response relationships, maternal and paternal identity are also determining the number of seeds produced (Bertin, 1990; Waser and Price, 1991; Holm, 1994; Melser *et al*., 1997; Mitchell, 1997; Bosch and Waser, 1999; Dieringer and Cabrera, 2002). In the present study a positive correlation was found between pollen viability and the number of seeds produced. This is in concordance with Hayase (1953) who stated that the seed number increased in proportion to the amount of viable pollen grains deposited on the stigma.

When the quality of seeds was assessed by their germination ability, the number of viable seeds were found increasing from initial phase to middle phase and decreasing to late phase of the day and season. According to Kevan and Eisikowitch (1990) cross pollination by insects increased the germinability of the resulting seeds in Canola (*Brassica napus* L.). Similar studies on cauliflower and cabbage (Partap and Verma, 1994) have shown that bee pollination enhanced the quality of the seeds of the crops. The present results indicate an increase in the percentage of viable seeds at increased number of viable pollen grains. The quality of the progeny may be influenced by the pollen deposited, through the action of prefertilization (Snow, 1986; Schlichting *et al*., 1987; Winsor *et al*., 2000) or postfertilization mechanisms (Niesenbaum and Casper, 1994; Rigney, 1995; Havens and Delph, 1996; Niesenbaum, 1999; Melser and Klinkhamer, 2001).

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