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Seed Dormancy, Storage Behavior and Germination of an Exotic Invasive Species, *Lantana camara* L. (Verbenaceae)

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Abstract

Lantana camara is a native shrub to West Indies. It has been introduced to several tropical and sub tropical countries. Now this plant has become invasive in these countries including Sri Lanka. The objective of this study was to gather basic information on seed biology of L. camara to aid in controlling it. Seeds were collected from numerous shrubs in Kandy and Ambalangoda. Seed moisture content (SMC), imbibition and germination of untreated and manually scarified seeds were determined. Effect of dormancy breaking treatments was studied. Length, width, mass, SMC and germinability of developing seeds were examined. Seed Moisture Content of L. camara (12.9%) suggested that seeds are orthodox. Both untreated and manually scarified seeds imbibed water in similar rate. None of the seeds germinated in light/dark and constant dark conditions. Further, seeds contained a fully developed embryo. Thus, it was concluded that L. camara seeds have physiological dormancy. Only a few seeds responded to dormancy breaking treatments suggesting that L. camara seeds have deep physiological dormancy. According to the ontogenical experiments, L. camara seeds attained physiological maturity and dispersal maturity by 4th and 5th week from pollination, respectively. It can be concluded that onset of dormancy occur before seeds attained physiological maturity, where germination of developing seeds was 0% at any stage. L. camara seeds showed the potential to retain in the soil seed bank. Thus, to control L. camara invasion, suitable methods have to be develop to deplete the soil seed bank.

Keywords: Lantana camara, seed dormancy, physiological dormancy, physiological maturity

Introduction

Exotic invasive species are introduced species beyond their natural habitats[1]. Invasive species are considered as the 2nd largest threat to the biodiversity of native communities[2]. Invasive species affect ecosystem process directly by decreasing abundance and richness of native species via competition, hybridization and predation; and indirectly, by changing the structure of the community and by changing the genetic diversity[3]. World's worst invasive species have been listed according to two criteria; illustration of important issues surrounding biological invasion and deleterious impacts on biodiversity and/or human activity. *Acacia mearnsii, Cecropia peltata, Rubus ellipticus, Mimosa pigra, Panicum maximum, Tamarix ramosissima* and *Lantana camara* are some of the examples for world's worst invasive species[1]. *Lantana camara* is a pantropical weed destributed over 60 countries worldwide[4]. The typical form is native to West Indies. Many of the countries and islands that were recorded as *Lantana* free countries in 1974 have been infested with *Lantana sp*. more recently due to increase of its distribution e.g. Galapagas island, Solaman islands, Palau, Saipan, Tinian, Yap and Futuna island[4].

L. camara was introduced to Sri Lanka in 1926 as an ornamental plant. It has widely distributed across the island and now it has become invasive[5]. Especially, it has invaded the Udawalawa National park, a leading elephant sanctuary in Sri Lanka[6] and now has become a threat to its fauna and flora[5].

Many management techniques such as mechanical removal, chemicals and biocontrol agents, have been used to control *L. camara,* though they were less effective[4]. Further, many research have been conducted to discover a successful biological control agent to control the spread of *L. camara*[7]. Since 1902, 41 different natural enemies of *L. camara* have been released in 42 countries[8]. In Australia, *Leptobyrsa decora, Phenacoccus parous* and *Prospodium tuberculatum* are used as biocontrol agents for *Lantana*[9]. However, most of the control strategies conducted was less effective[9]. Because *L. camera* has been developed as a hybrid ornamental plant, it is difficult to find a potential control agent. Further, *L. camara* has a high genetic diversity and it has a high hybridizing ability[10]. However, most of these eradicating programmes have been failed because these programmes were conducted without considering the biology of these invasive species.

Seed biology is an important aspect when considering the biology of invasive species. Information on seed biology (seed development, seed dormancy, seed storage behavior, seed dispersal, seed germination, allelopathic effects on germination, seed pathology and seed predation) are important in developing control strategies for invasive and weedy species[11;12]. Further,

the information about seed dormancy break and germination can be used to predict the future spread of weedy or invasive species[13]. Day et al.[4] have reported that the *L. camara* seeds have low germination rate both under laboratory and under field conditions. Graff (1987) proposed that seed dormancy and/or low seed viability may have caused low germination rate in *L. camara* seeds. Day et al[4] suggested that low germination rate of *L. camara* may be due to the meiotic instability. Raizada and Raghubanshi[2] have observed that the *L. camara* seed germination could increase by fire. When fleshy pulp was manually removed, germination rate of *L. camara* seeds increased from 10% to 46% and this rate is comparable to seeds collected from faeces of wild birds[4]. However, Raizada and Raghubanshi[2] claimed that even under favorable conditions, germination percentage of *L. camara* seeds is low (45%). If the low germination rate is due to the dormancy of *L. camara* seeds, invasion of *L. camara* is difficult to control using a mechanical control method, because dormant seeds can remain in the soil seed bank for longer time periods and can germinate under favorable conditions for several succeeding years.

Thus, the main objective of this study was to gather basic information on seed germination of *L. camara* to aid in controlling it. To fulfill this objective seed dormancy, storage behavior seed germination and seed development of *L. camara* have been studied.

Material and Methods

Study Species: *Lantana camara* (Verbenaceae) is a significant panatropical weedy shrub native to West Indies. This was introduced to many countries as an ornamental plant and still it is used as an ornamental plant in some countries[10]. It has been naturalized approximately 60 countries or island groups between 35 ^{0}N and 35 ^{0}S latitudes. It can be observed in waste areas, common along roadside fence rows and abandoned lands[4].

Collection of seeds: Seeds of *L. camara* were collected from numerous shrubs in July, 2010 and August, 2010 from two ecotypes: Kandy (23^{o}C - 29^{o}C, 2100 mm precipitation, 500 m asl.) and Ambalangoda (26^{o}C, 2400 mm precipitation, 0-20 m asl.). Seeds were stored in sealed polythene bags and brought to University of Peradeniya. Experiments were initiated within one week from the collection at the University of Peradeniya Sri Lanka.

Seed moisture content: Ten samples of five seeds from each collection were weighed initially using a digital chemical balance (JD 200-3) to nearest 0.001g. These samples were oven dried at 120 ^{0}C for 4 hours and reweighed[14]. Percentage seed moisture content was calculated using on fresh mass basis[15].

Characterization of the dormancy of *L. camara* seeds: ***Imbibition of seeds:*** Two samples of 15 manually scarified and non-scarified seeds were weighed initially using a digital chemical balance to nearest 0.001g at time 0. They were placed on moistened filter paper with distilled water in Petri dishes and kept at ambient laboratory temperature (≈ 28 ^{o}C) and light conditions (White florescent light and diffused light through windows). Seeds were removed at time intervals shown in the figure 1, blotted dry with filter papers, weighed and returned to the Petri dish.

Germination of non-treated or manually scarified L. camara seeds: Three replicates consisted with 15 untreated or manually scarified Kandy collected seeds were incubated in light/dark (ambient laboratory light conditions) and in constant darkness conditions at ambient laboratory temperature conditions (≈ 28 ^{o}C). Number of seeds germinated in light/dark was counted at 2-day intervals, while dark incubated seeds were checked for germination in 14-day intervals. Radical emergence was the criterion for germination. The same experiment was repeated for Ambalangoda collected seeds.

Determination of level of physiological dormancy in *L. camara* seeds: ***Breaking dormancy in intact seeds:*** Four samples of three replicates with 15 Kandy collected nontreated and manually scarified seeds were incubated on filter papers moistened with 100 ppm or 500 ppm gibberalic acid solutions. Samples were checked for germination in 2-day intervals. Radicle emergence was the criterion for germination. Manually scarified and non-scarified seeds that were collected from Ambalangoda were only subjected to 500 ppm GA treatment due to scarcity of seeds. Germination was checked in 2-day intervals.

Effect of storage on germination: Six samples of three replicates of 15 seeds were stored dry for 1, 2 or 3 months. Two samples of seeds were retrieved in one-month intervals. Seeds of one of these two samples were manually scarified. Seed samples were incubated under light/dark conditions in ambient laboratory temperature. Seeds were checked in 2-day intervals for germination. Radicle emergence was the criterion for germination.

Effect of Storage on gibberalic acid treatments: Twelve samples of three replicates of 15 seeds were stored dry for 2 or 3 months. Four samples of seeds were retrieved after storage. Seeds of two of these samples were manually scarified. Seed samples were incubated on moistened filter papers with 100ppm or 500ppm GA in light/dark conditions at ambient laboratory temperature. Seeds were checked in 2-day intervals for germination. Radicle emergence was the criterion for germination.

Germination of seeds buried in soil: One sample of 3 replicates of 100 seeds were placed in nylon mesh bags and buried approximately at 5-cm deep in the Department of Botany, University of Peradeniya premises. Bags were retrieved in one month intervals and checked for germination. Seeds were reburied after the check.

Ontogeny of seeds: ***Seed collection:*** *L. camara* developing fruits were collected in one-week intervals after pollination up to 35 days from a plant growing in Department of Zoology University of Peradeniya in September 2010.

Fruit length, width and weight: Fifteen developing fruits of *L. camara* were collected on 1, 2, 3, 4 and 5 weeks after pollination. Length and width of these fruits were measured to nearest 0.1mm using a millimeter ruler. Three replicates of 5 fruits or seeds were weighed to the nearest 0.001g in every week.

Seed moisture content: Three replicates of 15 seeds from 1, 2, 3, 4 and 5 weeks after pollination were weighed initially using a digital chemical balance (JD 200-3) to the nearest 0.001g. Samples were oven dried at 120 ^{0}C for 4 hours and reweighed[14]. Percentage seed moisture content was calculated based on fresh mass basis[15].

Germination of developing seeds: Three replicates of five non-scarified seeds from 1, 2, 3, 4 and 5 weeks after pollination were incubated on moistened filter papers in Petri dishes under light/dark conditions at laboratory temperature conditions (≈ 28 ^{o}C). Seeds were checked for germination in 2-day intervals.

Analysis of data: All the experiments were performed using a completely randomized design. Imbibition data was analyzed with a pooled t-test conducted using Excel software (version, Microsoft co-operation, USA). Results of the dormancy breaking treatments were analyzed using the two-way ANOVA procedure in the SAS statistical software (version 6.12, SAS institute Inc.cary, NC, USA). Data were normalized using Arcsine transformation prior to analysis. Duncan mean separation test was used to identify the differences between treatments**.**

Results and Discussion

Seed moisture content: Moisture content of seeds of *Lantana camara* was 12.9 ± 0.6 % on fresh mass basis.

Characterization of the dormancy of *L. camara* seeds: ***Imbibition of seeds:*** Both non-scarified and manually scarified seeds on moistened filter papers increased seed mass. Non-scarified seeds and manually scarified seeds imbibed water and 30% mass increment was observed within the first day (figure-1). In the fourth day non scarified seeds showed 30% - 40% mass increment, while manually scarified seeds showed 40% - 50% mass increment. Mass increment of manually scarified seeds and non-treated seeds were not significantly different from each other (T = 1.39, P = 0.175).

Germination of non-scarified and manually scarified seeds: Germination of non scarified seeds and manually scarified seeds incubated in light/dark or constant darkness were 0 % in seed collected from both Kandy and Ambalangoda within 30 days.

Determination of level of physiological dormancy in *L. camara* seeds: ***Breaking dormancy in intact seeds :*** Both non-scarified and manually scarified Kandy collected seeds that were incubated on 100 ppm or 500 ppm GA have germinated to a lower percentage. The highest germination percentage observed in Kandy collected seeds (18 %) was reordered from non scarified 500 ppm GA treated seeds, while the lowest germination percentage (2 %) was recorded from manually scarified 100 ppm GA treated seeds (figure-2). Highest germination percentage observed in Ambalangoda collected seeds, was recorded from 500 ppm GA treated manually scarified seeds (20 %); while the lowest was recorded in non scarified seeds treated with 100 ppm GA (15 %) (figure-3). No statistical difference was observed between the germination of seeds collected from two locations (F= 0.10, p=0.757).

Effect of storage on seed germination: Germination percentage of Kandy collected non-scarified and manually scarified seeds that were dry stored for 1, 2 or 3 months was 0%.

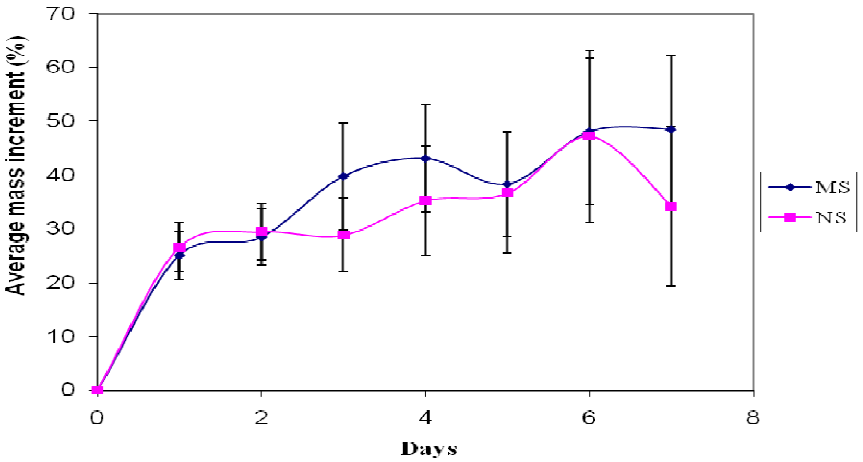

Figure-1
Average mass increment in non scarified and manually scarified seeds on moist filter papers at ambient laboratory temperatures (27 ^{o}C – 28 ^{o}C). Error bars = ± SE, Standard Error. MS, manually scarified seeds, NS, Fresh untreated seeds

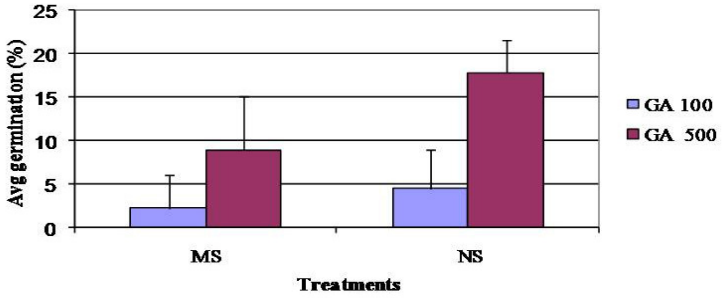

Figure-2

Germination of non scarified and manually scarified *L. camara* seeds collected from Kandy treated with GA (Gibberellic Acid) 100 ppm and 500 ppm concentrations within one month. Error bars = ± SD, Standard Deviation

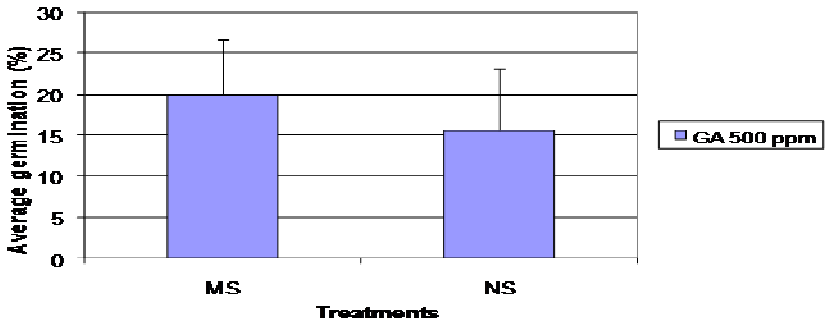

Figure-3

Germination of non scarified and manually scarified *L. camara* seeds from Ambalangoda treated with GA 100 ppm and 500 ppm concentrations within one month. Error bars = ± SD, Standard Deviation

Effect of storage on GA treatment: Kandy collected two months and three months dry stored seeds germinated to a lower percentage within the first month. The highest germination percentage (15%) of two months dry stored seed was recorded in manually scarified 500 ppm GA treated seeds while, the lowest germination percentage (2%) was recorded in both non-scarified and manually scarified 100 ppm GA treated seeds (figure- 4.). In three months dry stored seeds, the highest percentage germination (4%) was reordered in manually scarified 500 ppm GA treated seeds, while the lowest percentage germination (2%) was recorded in non-scarified 500 ppm GA treated seeds. There was significant effect in dry storage on average germination percentages ($F=6.07$, $p=0.0074$). Further, average germination percentage between two GA treatments (100ppm, 500ppm) were significantly different ($F=19.13$, $p<0.001$). No significant effect in seed scarification on seed germination ($F=0.00$, $p= 0.999$).

Germination of seed buried in soil: Germination percentage of buried seeds was 0 % throughout the 5-month experimental period.

Ontogeny of seeds: *Fruits length, width and weight:* Length, width and weight of developing *L. camara* seeds after 1 week from pollination was 2.8 ± 0.4 mm, 2.3 ± 0.5 mm, 0.06 ± 0.02 g respectively (figure- 5). After 4 weeks from pollination it was 5.7 ± 0.6 mm, 6.1 ± 0.7 mm, 0.6 ± 0.05g respectively. Developing fruit length, width and weight increased rapidly for four weeks and decreased gradually thereafter. At the end of the 5th week from pollination fruit length was 3.5 ± 0.5 mm (figure-5).

Seed moisture content: Fresh and dry mass of *L. camara* seeds increased for four weeks from pollination (figure- 6). After four weeks, fresh and dry masses of seeds were decreased. Moisture content of seeds, initially increased and highest moisture content (72%) was reordered in second week. After fourth week, moisture content was dramatically decreased.

Germination of developing seeds: Germination percentage of non-scarified seeds collected from 1, 2, 3, 4 and 5 weeks after pollination, under light/ dark conditions were 0%.

Moisture content (13 %) of fresh *Lantana camara* seeds suggests that they are orthodox 1. Therefore, *L. camara* seeds can survive under low moisture contents and could have a long storage life[1]. Both non scarified and manually scarified seeds of *L. camara* imbibed at a similar rate and no significant difference was observed in final mass increment. Thus, it indicates an absence of water impermeable layers in the seed/fruit coat i.e., *L. camara* seeds have no physical (PY) or combinational (PY +PD) seed dormancy[17]. The results of the germination test suggest that *L. camara* seeds have dormancy where only few seeds germinated during the time of experiment. This is in

agreement with the results of Graff[18] where he proposed that low germination rate in *L. camara* seeds was due to seed dormancy. However, he has not shown the data on which he came to this conclusion. Day et al.[4] claimed that when the fleshy pulp of the fruit was manually removed germination rate increased from 10 % to 46 %. Thus, their results also revealed *L. camera* seeds have seed dormancy. However, in our experiments even the fleshy exocarp removed seeds germinated to a lower percentage. *L. camara* seeds contain fully developed embryo i.e. embryo fill the whole seed. Therefore, it can be concluded that seeds have no morphological dormancy (MD) or morphophysiological dormancy (MPD). Thus, it can be suggested that seeds of *L. camara* may have physiological dormancy (PD). Seed dormancy of a small portion of *L. camara* seed sample was alleviated with GA treatments. GA can assist in dormancy break by increasing the growth potential of the embryo and can also weaken the tissues surrounding the radical[19]. Germination of seeds with non deep and intermediate physiological dormancy is increased with GA treatments[11,20-22] (whilst it does not promote germination of seeds with deep physiological dormancy[11]). During our experiment, only a low germination rate of *L. camara* was recorded even after the GA treatment. This suggested that *L. camara* seeds have deep PD. None of the *L. camara* seeds germinated after two and three months of dry storage at ambient laboratory conditions. Dry storage breaks the dormancy of seeds with non deep or intermediate PD[11]. In our experiments, 2-month dry storage increased the sensitivity of *L. camara* seeds to the 500 GA treatment. Dry storage increase the sensitivity to GA treatments of physiologically dormant seeds. This condition is in agreement with seeds with deep physiologically dormant seeds. Thus, we can conclude that the seeds of *L. camara* have deep PD. Most of the Verbenaceae species produce seeds with PD[23-27], while some of the species produce seeds with nodormancy[28].

Baskin and Baskin[11] proposed to keep seeds in their natural environments and monitor for germination to get an understanding about the seed dormancy breaking treatment of them. However, in seeds which buried in the Department of Botany, University of Peradeniya premises, with high soil moisture content have not germinated during the 5 month experimental period. Species specific environmental cues are required to break the seed dormancy[11]. Thus, buried seeds may not have received the dormancy breaking cue during the burial period from October 2010 to March 2011. Some weed seeds have an ability to remain viable for about 100 – 600 years[15] until their requirements for germination or dormancy break (such as light, alternating temperature, mechanical disturbances or abrasion) are fulfilled. Dormancy break is influenced by diurnally fluctuating temperatures and it is linked to soil seed bank persistence[29]. Species maintaining long-term persistent seed banks can play a role in re-colonization of abandoned lands[30]. Under natural field conditions seed persistence in the soil is an important factor for the maintenance of local plant populations[29].

The three distinct seed development phases can be identified during the development of *L. camara* seeds. In the first phase of *L. camara* seed development (P1), there was no change in the moisture content. This is the phase where embryo and endosperm development occur. In the second phase (P2) moisture content decreased (from 71.88% to 47.78%) slowly in *L. camara* seeds. According to Le Deunff & Rachidian (1988) this is the phase where cotyledons and endosperms are filled with storage materials. According to our results, dry mass of *L. camara* seeds increased during four weeks and the highest gain of dry mass recorded at fourth week after pollination. Thus, we can conclude that *L. camara* seeds attained physiological maturity 4 weeks after pollination[32]. During the third phase seed moisture content decreased dramatically from 47.78% to 14.92%. According to the external appearance of *L. camara* fruits, they attended dispersal maturity in the fifth weeks after pollination. After the fifth week from pollination, fruits fall down from the shrub. Generally onset of dormancy occurs during the time period between physiological maturity and dispersal maturity. Thus, if there is no dormancy, most seeds can germinate before the physiological maturity[33]. However, none of the developing *L. camara* seeds collected 1, 2, 3 or 4 weeks after pollinated germinated, i.e., onset of physiological dormancy has occurred before physiological maturity stage.

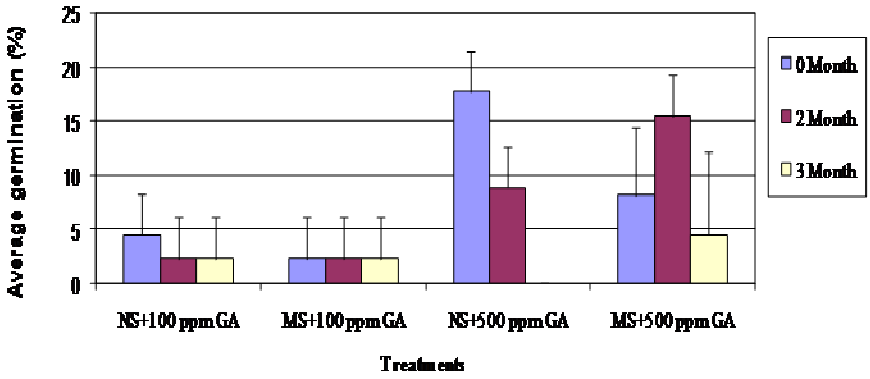

Figure-4

Germination of non scarified and manually scarified *L. camara* seeds treated with GA 100 ppm and 500 ppm concentrations after dry storage in 2 and 3 months. Error bars=±SD, Standard Deviation. MS, manually scarified seeds; NS, untreated seeds

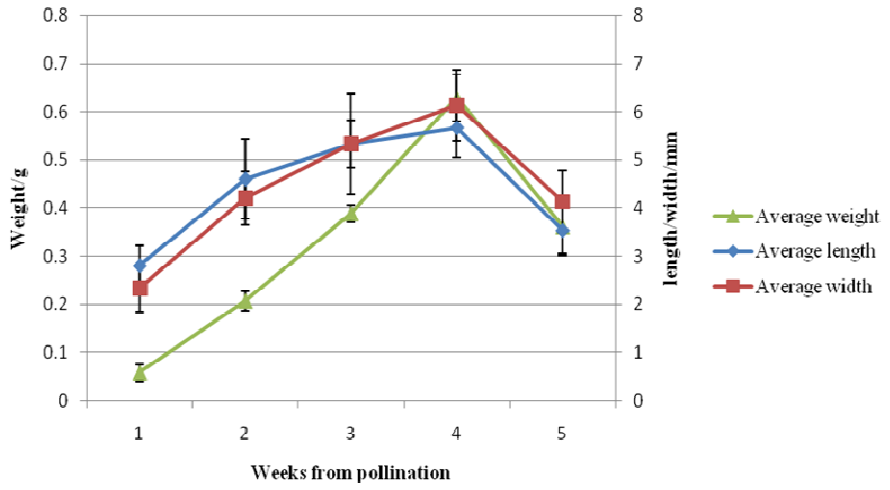

Figure-5
Length, width and weight during development of *L. camara* fruits. Error bars = ± SD, Standard Deviation

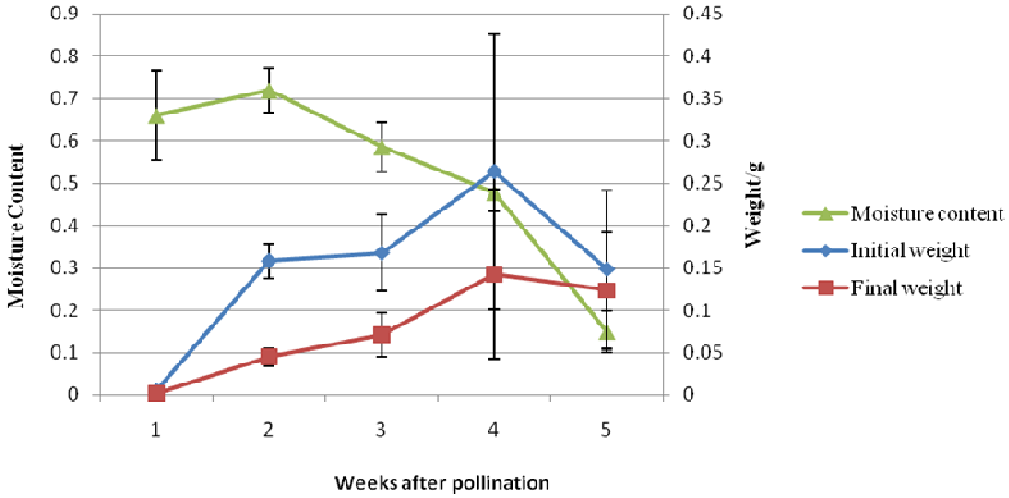

Figure-6
Fresh mass, dry mass and moisture content (in fresh massbasis) of *L. camara* seeds 1, 2, 3, 4 and 5 week after pollination. Error bars = ± SD, Standard Deviation

Conclusion

Distribution of *L. camara* is still increasing in many countries and islands world wide[10]. Many control strategies have been used to control this plant. Some of these methods are physical harvesting, chemical and biological controls methods. However, most of these methods were less effective[9]. According to our study *L. camara* seeds have deep physiological dormancy and seed storage behavior of them is orthodox. Thus, *L. camara* seeds have the capacity to produce a long- term persistent soil seed bank. Therefore, apart from the physical removal of the plants we recommend to incorporate measures to deplete the soil seed bank to control *L. camara* invasion. Our research has opened some windows to conduct further studies on the seed dormancy of *L. camara* rather than providing a blind end conclusion. Although, fresh seeds and stored seeds have high level of dormancy (according to the current research). Day et al.[4] recorded that seeds collected from faeces of wild birds show the high germination rate. Thus, it is important to study this phenomenon further to understand the significance of bird dispersers on breaking dormancy of this species. Most seeds with deep physiological dormancy require cold stratification to come out of dormancy[11,34,35,36,37]. However, *L. camara* is a tropical and sub-tropical species which naturally do not receive cold stratification. Thus, we have not conducted experiments to reveal the effect of cold stratification on seed dormancy break of *L. camara*. As warm stratification has not successful in breaking dormancy of this species, to understand the natural dormancy breaking conditions seed burial experiment can be continued for at least 2 years. Further, as suggested by Baskin and Baskin[26] move-along experiment can be used to identify the dormancy breaking temperature of seeds of this species.

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