

Determination of Optimum Mulberry Silkworm Larvae Bed Spacing on Feeding Tray using Shelf Rearing Technique

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Abstract
Bed spacing is an important silkworm rearing practice to ensure the hygiene of silkworms in order to protect from disease infection and to ensure them good feeding appetite. Different stages of silkworms require different bed space. As worms increase in size, there is overcrowding and overlapping each other that leads to underfeeding, creating a microclimate for disease spread and could also lead to suffocation. Hence, appropriate bed spacing is essential to silkworms, to keep the worms healthy and productive. The treatments used for examining the appropriate bed spacing of young and medium aged silkworm larvae were 1200, 1000, 800, 600, 400 and 200 per feeding tray (60cm X 90cm size). Another arrangement of bed spacing used for examining the mature aged silkworm larvae was 600, 500, 400, 300, 200 and 100 per feeding tray (60cm X 90cm size) using silkworm shelf rearing technique to evaluate the effects of mulberry silkworm bed spacing on different silkworm strains.1st and 2nd larval instars as young age, 3rd and 4th larval instars as medium age, 5th larval instar as mature age were considered in the study. Observations on larval mortality, single larval weight, single shell weight, and silk ratio from different silkworm strains were carefully noted for each treatment and replications. Three replications were used for each treatment. Statistically significant variation in mean larval mortality rate among bed spacing was observed in young, medium and mature silkworm larval stages of silkworm strains. Larval mortality rate was significantly ($P < 0.05$) reduced when young, medium and mature larval stages of mulberry feeding bivoltine silkworm strains (Korean bivoltine and Kenyan bivoltine silkworm strains) reared in a group of 400 to 1000 worms, 400 to 800 worms and 300 to 500 worms respectively in a 60 cm X 90 cm feeding tray of shelf rearing method. A significant ($P < 0.05$) reduction of larval mortality was recorded from mulberry multivoltine Vietnamese silkworm strains in a bed spacing of 400 to 1200 larvae for young larval stages, 400 to 1000 larvae for medium larval stages and 300 to 600 larvae for mature larval stages. Bigger Larval weight, bigger shell weight and higher percentage of silk ratio are important cocoon yield quality parameters for silkworm strains. Significant ($P < 0.05$) variations were observed among population densities for these important variables in all the tested silkworm strains.
Keywords: bed spacing, silkworm strains, feeding tray, larval instars, larval mortality, shell weight, silk ratio

Introduction

Sericulture is the process of obtaining the natural silk fiber through silk worm rearing, which can be practiced in varying agro-climatic conditions, and is suited to different production systems (Singh *et al*., 2002). Ethiopia is granted with diversified climate, vegetation and topography. This is also true for diversified options of sericulture industry which are adopted on different vegetation (for feeding of silk-worms) and different species of silkworms (Metaferia *et al*., 2006). FAO (1976) published that silk production is a highly profitable business enterprise that can produce good return from small portion of land where most of the land can be used for other food crops production. Further, the by-products also find uses ranging from fertilizers in rural areas to pharmaceutical industries. Thus, silk production has the potential to make a significant contribution to the economy of many countries where there is surplus labor, low-costs of production and a willingness to adopt new technologies (Hajare *et al*., 2007). The business holds a ray of hope at village level for Ethiopian citizen migrating to cities searching for jobs **(**Kedir *et al*., 2014**)**. Rearing performance in silkworms is affected by ecological, biochemical, physiological and quantitative characters, which influence growth and development, quantity and quality of silk they produce in different geographical locations Ramesh *et al*, 2012; and Reddy *et al*, 2012). The success in silkworm rearing depends on the various factors including successful implementation of technological and managerial tools along with high yielding and best-suited feed plant varieties and silkworm strains (Rajan and Himantharaj, 2005). Silkworm rearing starts from egg stage and terminating in adults laying eggs and dying their natural death. During this course, they pass through five larval instars (1st, 2nd, 3rd, 4th, and 5th instars) intervened by four moults, cocoon and pupal stage (Singh *et al.,* 2002). Silkworm rearing effectively means the culturing of five larval instars with better management practices. During larval growth stage bed cleaning (Abiy *et al*, 2015) and spacing is an important silkworm rearing process to ensure the hygiene in the immediate vicinity of silkworms in order to protect from disease infection and to ensure them good feeding appetite (Gogoi and Goswami 1998). As soon as the larvae grow-up, the size increases in the rearing bed which ultimately cause changing atmosphere and favoring multiplication of pathogenic organisms such as protozoa, fungi, bacteria and viruses (Sannappa *et al*.,1999). Hence, an appropriate spacing should be done for good growth and feeding to protect them from fungal, bacterial and viral infections (Sannappa *et al*., 1999). Diseases are the behavioral and physiological changes induced by pathogens in an organism (Hisao, 2001). If proper bed spacing is not done according to larval ages it leads to various complications viz. ill health of larvae, disinterest of the larvae to feed due to unhygienic conditions, ultimately worms becomes weak and low in productivity (Hisao, 2001).Since they cause substantial financial loss to the silk producers, their prevention and control assumes utmost importance. Hence, appropriate bed spacing is essential to keep the worms healthy and productive. Bed spacing studies revealed that when larvae were densely populated more number of dead larvae was observed compared to sparsely populated once (Zhang,*et al.,* 2002). They further revealed that appropriate bed spacing minimizes the larval mortality. Sakthivel (2004) observed superior larval growth, development and higher cocoon production when mulberry silkworms were properly placed on feeding trays according to their age. The silkworm culture adaptation is being practiced in a large scale on the leaves of mulberry with ideal spacing to administer nourishment to all the worms simultaneously and thereby to secure uniform growth and development of the worms (Neelu *et al*.,2000). They further mentioned that next to feeding, spacing and bed cleaning is an equally important factors. It is necessary for the health and progress of the worms. Sharma *et al*., (1996) also observed that silkworms at proper spacing resulted in significantly higher larval weight, larval survival, cocoon weight, shell weight, shell ratio, pupal weight, rate of pupation, silk productivity, fecundity and egg hatching with lower larval and pupal durations than those raised under improper condition in farmers' practices. The present study was therefore conducted to determine stage wise bed spacing of the different mulberry silkworm strains reared at Melkassa sericulture laboratory. The study also sought to examine the effect of bed spacing on larval mortality, larval weight and yield components of the different mulberry silkworm strains.

Materials and Methods

This study was carried out at Melkassa Agricultural Research Center (MARC), sericulture research laboratory in 2014 to 2016. As per the rearing recommendations of silkworms by Rajan and Himantharaj (2005), the silkworm rearing room and equipments were cleaned, washed and disinfected with 2% formalin solution at the rate of 800ml per $10m^2$ before the commencement of the experiment. The silkworm breeds were reared following shelf rearing techniques starting from brushing till cocoon spinning. Silkworms at larval stage were fed four times a day with tender leaves until 2^{nd} instar, semi tender leaves to 3^{rd} instar larvae and mature leaves until 5^{th} instar. For each feeding tray equal amount of feeds from same feed plant variety were given. Mulberry (*Bomyx mori*) plant was cultivated and used as feed source for these silkworms. The grown up worms, after completing feeding during late fifth instar at their ripened stage (ready to spin silk) were picked and transferred on the mountages (equipment to provide support for cocoon formation) for spinning silk cocoons. Ripened silkworms were identified by their characteristics movement to the corners of the rearing beds, reduction in size and transparent yellow appearance. On the sixth to eighth day of spinning, the cocoons were harvested, counted and weighed (Singh and Benchamin, 2002).The experiment was arranged in Completely Randomized Design (CRD) in three replications. Observations on larval mortality rate, larval weight and qualitative characters of the cocoon (cocoon shell weight and silk ratio) were recorded. Different mulberry silkworm strains Viz…,Kenyan bivoltine, Korean bivoltine and Vietnamese multivoltine strains were evaluated in the experiment. The treatments used for examining the appropriate bed spacing of young and medium aged larvae were 1200, 1000, 800, 600, 400 and 200 silkworm larvae per feeding tray (60cm X 90cm). Another arrangement used for examining the appropriate bed spacing of late or mature aged larvae were 600, 500, 400, 300, 200 and 100 silkworm larvae per feeding tray (60cm X 90cm) for silkworm shelf rearing technique. Complete randomized design with three replications was used for each treatment. Statistical analysis software (SAS) was used to analyze the data using analysis of variance (ANOVA) procedure. Least significant difference (LSD) was used for mean separation. Percentage proportions were calculated for larval mortality rate.

Results

Effect of bed spacing on larval mortality of different silkworm strains:

This study attempted to understand the effect of bed spacing on mortality of young (1^{st} and 2^{nd} larval stages), medium (3^{rd} and 4^{th} larval stages) and mature stages (5^{th} larval stage) of silkworm strains introduced from different countries.

Kenyan bivoltine silkworm strains: Significantly lower young aged larval mortality rate (0.817% to 1.583%) was recorded from a group of 400 to1000 worms reared per feeding tray. In medium larval stages, lower number of larvae were registered from a density of 400 to 800 worms (0.896 to 1.139%) and higher number of dead larvae was found from a population density of 200, 1000 and 1200 worms per feeding tray with larval mortality of 2.667%, 1.766%, 2.309% respectively. when mature larval stages reared in a density of 300 to 500 worms the mortality rate was significantly reduced to 4.75%. larval mortality was significantly increased when reared in a group of 100 worms (15.33%), in a group of 200 worms (8.33%) and in a group of 600 worms (10.331%) .

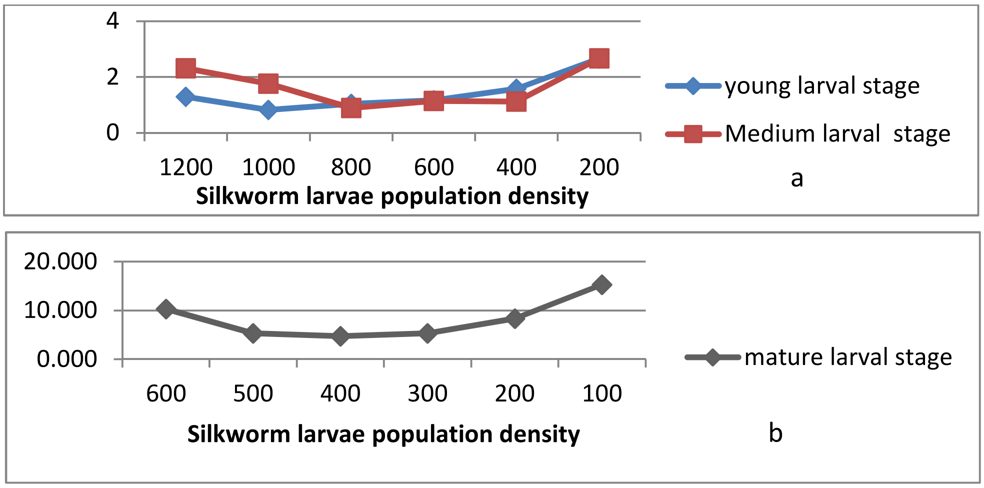

Figure 1. Effect of silkworm larvae bed spacing on mortality of young, medium and mature larval stages of Kenyan bivoltine silkworm strains

Korean bivoltine silkworm strains: Statistically significant variation in mean larval mortality rate among bed spacings was observed in young, medium and mature silkworm larval stages. An appropriate bed spacing/population density was found when young larval ages reared in a group of 400 to 1000 worms in a 60cm X 90 cm rearing tray in shelf rearing method where by the worms' mortality rate was not exceed a maximum of 0.833%. In the same age group significantly higher mortality was obtained from a density of 1200 worms with a death rate of 2.25%. For medium larval age group, a convenient bed spacing of 400 to 800 worms per feeding tray was found with a mean maximum death rate of 0.125%.

For mature larval age group, an ideal bed spacing of 300 to 500 worms per feeding tray was found with a mean maximum death rate of 0.667%. When mature worms are reared in a group of 600 in number, significantly higher percentage of larval mortality (6.33%) was recorded for the same strain.

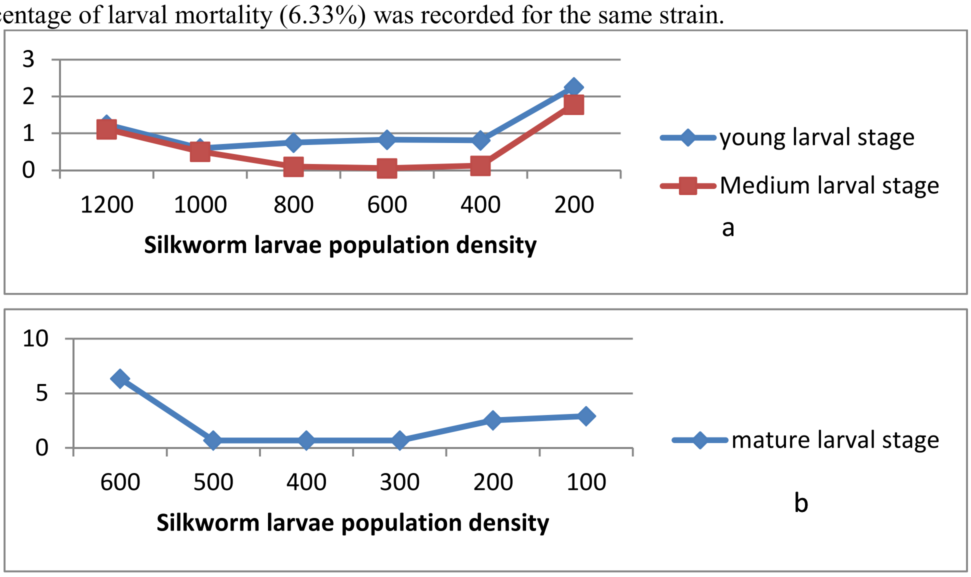

Figure 2. Effect of silkworm larvae bed spacing on mortality of young, medium and mature larval stages of Korean bivoltine silkworm races

Vietnamese multivoltine mulberry silkworm strains: For rearing young larval stage of this silkworm strain, larval mortality rate was reduced significantly in 400, 600, 800, 1000 and 1200 worms treated beds. The mortality rate was ranged from 0.506% to 0.764%. Treated beds with 200 worms showed significantly higher larval death rate (1.278%) for the same larval strain. For medium larval age groups, rearing beds treated with 400, 600, 800 and 1000 worms showed a significant lower mortality rate which ranged from 0.078% to 0.123%. When a density was used in1200 and 200 worms per feeding tray mortality rate were higher with 0.894% and 0.361% respectively. For mature larval age groups, a significant lower death rate was recorded from 300 to 600 worms placed per rearing tray. In these treatments mortality rate was ranged from 0.25% to 0.321%. When the density was 100 and 200 worms significantly higher mortality rate was registered. This was 1.22% and 1.20% respectively.

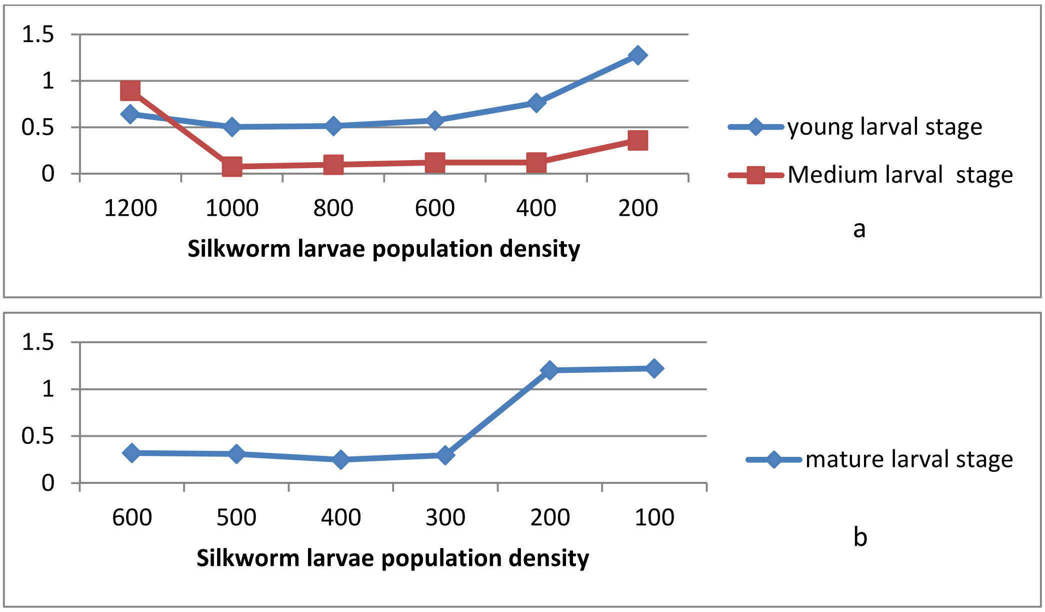

Figure 3. Effect of silkworm larvae bed spacing on mortality of young, medium and mature larval stages of Vietnamese mulberry multivoltine silkworm strains

Larval Weight: larval weight was significantly (P < 0.05) increased when 100 to 500 worms grown per feeding tray for Kenyan, (3.09 grams to 3.13 grams), for Vietnamese multivoltine, (2.18 grams to 2.21 grams) and for Korean bivoltine silkworm strains (3.24 grams to 3.29 grams). The least larval weight for all strains was significantly obtained from a density of 600 larvae grown per feeding tray.

Table 1. Mean effects of silkworm larvae bed spacing on larval weight (gm) in different silkworm strains

population density	Kenyan bivoltine	Korean bivoltine	Vietnamese multivoltine
600	2.63c	2.51b	1.43b
500	3.1b	3.24a	2.18a
400	3.11ba	3.24a	2.18a
300	3.12ba	3.26a	2.2a
200	3.09ba	3.28a	2.2a
100	3.13a	3.29a	2.21a
CV	0.613	4.82	0.997
LSD	0.033	0.275	0.037

Effect of silkworm larvae bed spacing on qualitative characters of the cocoon of silkworm strains:

In this study qualitative traits of the silk cocoon (silk shell weight and silk ratio) were also evaluated to the different bed spacing treatments. Table 2 summarizes the mean values of qualitative characters of the silk cocoon after the 5th larval instar was treated to the different bed spacings/population densities. This study revealed that there was statistically significant difference in shell weight and silk ratio among population densities in all silkworm strains (Table 2). Bigger shell weight was significantly (P < 0.05) obtained in a population density of 100 to 500 worms placed in one feeding tray for Kenyan bivoltine silkworm strains (0.373g to 0.396g) and for Vietnamese multivoltine (0.290g to 0.306g). Significantly bigger shell weight was registered for Korean bivoltine (0.373g to 0.413g) from 100 to 400 silkworm population densities while 500 to 600 worms in a feeding tray had small shell weight for this silkworm strain. A significant small shell weight was recorded in all silkworm strains while placing 600 worms in one feeding tray (silk worm bed). Higher percentage of silk ratio was achieved when a group of 100 to 500 worms are placed in a feeding tray from Kenyan bivoltine (22.0% to 22.98%), from Vietnamese multivoltine (22.36% to 22.86%) and from Korean bivoltine (21.6% to 24.2%). This study clearly showed that, percentage of silk ratio was significantly lower in all silkworm strains from a group of 600 worms reared in one feeding tray.

Table 2. Mean effects of silkworm larvae bed spacing on silk shell weight (grams) and silk ratio (%) in mulberry silkworm strains

population density	**Kenyan bivoltine silkworm** strains		**Korean bivoltine silkworm** strains		**Vietnamese multivoltine silkworm strains**	
	silk shell weight (gram)	silk ratio (%)	silk shell weight (gram)	silk ratio (%)	silk shell weight (gram)	silk ratio (%)
600	0.310±0.015b	18.773±0.428c	0.307±0.018c	19.75±0.43c	0.246b	18.713b
500	0.373±0.012a	22.036±0.159ba	0.337±0.013bc	21.76±0.57b	0.306a	22.400a
400	0.376±0.008a	21.653±0.384b	0.380±0.011ba	22.04±0.57b	0.300a	22.466a
300	0.376±0.003a	22.426±0.247ba	0.413±0.024a	24.25±0.57a	0.290a	22.356a
200	0.376±0.006a	22.880±0.376a	0.373±0.018ba	21.60±0.15b	0.306a	22.856a
100	0.396±0.003a	22.976±0.486a	0.393±0.012a	22.63±0.32b	0.296a	22.810a
CV	4.026	2.927	8.103	3.413	5.120	2.536
LSD	0.027	1.160	0.054	1.366	0.027	1.012

Discussion

The success of sericulture industry depends upon several factors of which the impact of the silkworm management practices is of vital importance. Among the management practices, silkworm bed spacing plays a major role on growth and productivity of silkworm (Benchamin and Jolly, 1986). Literatures stating that good quality cocoons are produced through applying appropriate silkworm bed spacing and significant deviations from these levels make the cocoon quality poorer. In the present study, performance of silkworm strains was tested in different bed spacing which generally proved a minimum and maximum population density in a range of 400 to 1200 worms for young larval stages, 400 to 1000 worms for medium larval stages, 300 to 600 worms for mature larval stages respectively depending on the type of silkworm strains. The different silkworm population density showed statistical significant differences among themselves in terms of important silkworm characters and silkworm mortality rate at $p<0.05$. Related studies conducted by Shah *et al*. (2007), showed variations in the performance of silkworms at different bed spacing levels. Moreover, significant variability among different bed spacing levels on silkworm mortality (0.056% to 15.33%) were recorded which was also in conformity with Qader *et al* (1992) who observed such differences among groups of population density through mortality rate studies. In regard to cocoon parameters, this study showed variations in single shell weight (0.310 gram to 0.413 gram) in mulberry feeding bivoltine silkworms, (0.246 gram to 0.306 gram) in mulberry feeding multivoltine silkworms and silk ratio (18.77% to 24.25%) in mulberry feeding bivoltine silkworms, (18.71 to 22.85%) in mulberry feeding multivoltine silkworms. This result is in agreement with findings of Nguku *et al* (2009) who studied the performance of different silkworm strains at different bed spacing levels. In general, all differences could be justified because rearing performance in silkworms is affected by silkworm population density which influence growth and development, quantity and quality of silk they produce in different geographical locations (Ramesh *et al*, 2012; and Reddy *et al*, 2012). Among silkworm management determined factors, Scriber and Slansky (1981) stated that bed spacing in the range of 400 to 1000 worms for young larval stages, 400 to 800 worms for medium larval stages, 300 to 600 worms for mature larval stages depending on silkworm breeds are required for silkworms effective growth and cocoon productivity.

It is known that the productivity of silkworms is affected by bed spacing and is verified in the silkworm strains reared. In addition, the silkworm mortality rate and silk cocoon traits vary to contribute to variability in performance of silkworm strains at different groups of population density. (Jayaramiah and Sannappa, 1998). Sannappa *et al*., (1999) reported that as soon as the larvae grow-up, its size increases in the rearing bed which ultimately causes feed competition and suffocation. They further mentioned that due importance towards good silkworm management practices in and around the rearing house are a prerequisite for a successful cocoon harvest and ideal rearing condition such as bed spacing should be done as per the different instars of larval stages and silkworm breeds. This study also confirmed there was variability in larval mortality rate at young, medium and mature silkworm larvae of different silkworm strains in respective with various bed spacing treatments. The results showed that using un ideal bed spacing caused significant mortality rate due to suffocation and feed competition in all silkworm strains. Rearing under better feeding, bed cleaning and bed spacing ensuring pathogen free rearing conditions are some of the vital requirements for the growth of healthy worms. This enables the silkworm tolerate adverse conditions. Using appropriate bed spacing according to larval size and weight ensures good bed hygiene, prevention of diseases and good larval growth (Sannappa *et al*., 1999). Devaiah *et al*., (1985) reported that feeding, spacing and cleaning are important silkworm management practices affecting the larval weight, silk gland weight, cocoon weight and shell weight considerably. In our study the cocoon characters after the 5th instar was treated in different bed spacing treatments providing supportive evidence for good rearing condition, also statistically significant among the tested population densities on many parameters. Bed spacing experiment revealed that some treatments were statistically superior in terms of minimum larval mortality, bigger larval weight, bigger silk shell weight and highest silk ratio depending on the successive larval stages of silkworm strains (Figures 1 to 3 and Tables 1 and 2). After successful silkworm rearing such as ideal bed spacing, cocoon weight gain was recorded in previous findings. This difference in shell weight gain may be attributed to the difference in the bed spacing treatments selected for the study (Table 2). Results from our study revealed that variations in larval mortality rate, larval weight, shell weight and silk ratio were obtained in the treatments in all silkworm strains. The findings of our study are in agreement with those of Joshi and Misra (1982); Hajarika *et al*., (2003). They found that higher effective rate of rearing, cocoon weight, shell weight and shell ratio were achieved using ideal bed spacing treatments. This was achieved by integration of not a single but a multitude of approaches viz. proper disinfection of the rearing room and appliances, use of bed disinfectant as per recommendations (Rajan and Himantharaj (2005), bed cleaning (abiy *et al*., 2015) and feeding (Ahmed *et al*., 2017). Prevention is better than Cure" is the correct approach and that should be adopted in integrated silkworm rearing management (Nataraju *et al*., 2005). This means that one should go about actively providing good silkworm management practices before any problem occurs, and it is only when preventive measures are in force that we can hope to effectively increase silk production and productivity. Patil *et al*., (2009) observed that silkworms receiving ideal management practices such as bed spacing, bed cleaning and feeding showed significantly higher larval weight (3.30 g), effective rate of rearing (90.0%), shell weight (0.475 g), shell ratio (23.02%), with lower larval and pupal durations in mulberry feeding silkworms. Reddy *et al*., (1989b) recorded survival rate (95.67%), silk ratio (22.00%), and shorter developmental period (26.49 days) when mulberry feeding bivoltine silkworms were reared on ideal bed spacing treatment. In Our study results indicating that the maximum shell weight (0.477 g) and silk ratio (22.97%) have been obtained in mulberry feeding silkworms' shelf rearing method. Devaiah *et al*., (1985) reported that feeding, bed spacing and bed cleaning are important worm management practices affecting the larval weight, silk gland weight, cocoon weight and shell weight considerably. According to them maximum larval weight (3.45 g), effective rate of rearing (92.0%), shell weight (0.48g) and silk ratio (22.5 %) has been obtained in shelf method of rearing.

Conclusion

Bed spacing or population density during the larval growth at different stages is one of the crucial issues for successful cocoon crop of silkworm strains. The silkworm strains studied differ in their optimum larval population density requirements during their growth. Statistically significant variation in mean larval mortality rate and silk cocoon traits among population densities was observed in young, medium and mature silkworm larval stages. Different stages of silkworms require different bed space. Overcrowding of medium and mature silkworm larvae in a rearing bed/tray leads to underfeeding, creating a microclimate for disease spread and could also lead to suffocation. The larval development will be maximum during the 5th Instar after 4th moult. Too much crowd in the rearing tray results in increase in humidity, heat, fermentation of litter which results in un-hygienic conditions, wastage of leaf and under development of silkworm while sparse or low population of young silkworm larvae in a rearing bed/tray is loss of space. So, appropriate spacing should be given simultaneously at the time of bed cleaning or feeding and care should be taken not to disturb the worms very frequently.

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