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Evaluation of multivoltine x bivoltine hybrids of mulberry silkworm, Bombyxmori L. tolerant to disease and high yielder at various generations for end users

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The trial was conducted at Melkassa research center in the sericulture research laboratory. Four bivoltine and one polyvoltine silkworm were involved in the crossing experiment and laid out in complete randomized design with four replications. Data was collected on fecundity, pupation rate (%), number of diseased larvae, survival rate (%), cocoon weight (g), cocoon shell weight (g), cocoon shell ratio (%) and filament length (m). Uniform and non-significant numbers of eggs produced by adults and cocooning percentages were recorded for both hybrid and parents in all generations. Average larval weight significantly (P<0.01) lower for F1, F2, F3 and F4 generations hybrids than parents but higher in F5, F6 and F7 generations hybrids. Silk ratios and survival rates significantly (P<0.01) higher for all hybrids than parent bivoltine in all hybrid generations. Larval period significantly (P<0.01) lower in F5, F6 and F7 generations hybrid than parent bivoltines. Filament length significantly (P<0.01) lower in F1, F2, F3 and F4 generations hybrids than parent bivoltine but significantly higher in F5, F6 and F7 generations hybrids than parent bivoltines. It can be concluding that instead of using parent polyvoltine and bivoltine mulberry silkworms separately for silk production; the farmers can use F5 and above generations hybrids of multivoltine x bivoltine for relatively higher diseases resistant and maximum silk productions.

Key: Polyvoltine silkworm, bivoltine silkworm, hybrids

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INTRODUCTION

Silkworm diseases form major constraint in realizing full potential of the silkworm hybrids. Among all the silkworm diseases that cause damage, viral diseases are most serious (Samson et al., 1990; Subba et al., 1991; Sivaprakasam and Rabindra, 1995). Nuclear polyhedrosis (BmNPV) belongs to Baculoviridae, causes nuclear polyhedrosis (grasserie) in silkworms which is most common viral disease and is prevalent in almost all the sericulture areas in India. Grasserie disease accounts for about 50% of total crop loss due to viral diseases (Samson, 1992).

Under above circumstances, among many measures of silkworm disease control and prevention, the utilization of disease resistant/tolerant silkworm breed/hybrid along with the disinfection would be the most effective step in the direction of the disease prevention (Sivaprasad and Chandras hekharaiah, 2003). Breeding as an important tool has been used by many breeders for exploiting the inherent heterosis. The aim of the most breeding programmes is to improve the yield potential of the breeds/hybrids over the existing which has played a vital role in increasing the productivity in sericulture (Reddy et al., 2008, 2009a-c, 2010a; Seshagiri et al., 2009**).** Silkworm hybrids show improved reeling performances over pure races (Reddy et al., 2010b, 2009c). The silkworm *Bombyxmori* L. forms one of the very important insects of choice with large number of strains that is best exemplified for utilization of heterosis by crossing them in different combinations (Datta and Nagaraju, 1987).

Among various reasons for low productivity of Ethiopian silk worms are , the lack of highly productive silkworm and diseases resistant races suitable to environmental conditions. In addition, Ethiopian sericulture is mostly polyvoltine and some bivoltine oriented and the qualities of the breeds have deteriorated as a result of continuous and prolonged inbreeding. Thus, breeding emphasizes the need for developing promising genotype of known genetic potential to increase the productivity in plants and animals (Yokoyama, 1956).

Melkassa Agricultural Research Center started sericulture activity since 1990 on Eri (eri-3.4 and India) and Mulberry silkworms (multivotine and bivoltine). For the last 14-15 years the silkworms were maintained in the center through self crossing methods. As the result, disease tolerant capability is deteriorating from time to time for some races of Eri and mulberry silkworms due to losses of resistant genetic potential, while the others are relatively resistant to disease and high yielder. Inter-strain/breed differences in susceptibility or relative tolerance to a number of silkworm diseases have been reported (Sivaprasad and Chandrashekharaiah, 2003).

However, no report was available on development of multivoltine x bivoltine silkworm hybrids tolerant to diseases, especially under Ethiopian tropical conditions. Thus, in the present study an attempt was made to develop relatively higher diseases tolerant and yielder cross breed of silkworm for commercial as well as farmer's exploitation.

OBJECTIVES

- To develop relatively disease resistant hybrids of Mulberry silkworm, *Bombyxmori*
- To develop high yielding hybrids of Mulberry silkworm, *Bombyxmori*

MATERIAL AND METHODS

The trial was conducted at Melkassa Agricultural Research Center, in the sericulture research laboratory.

By employing multivoltine x bivoltine mulbery feeding silkworms crossing and a total of four hybrids and five parents were used in the experiment (Mulberry multivoltine(mmyc) x Mulberry bivoltine (Kenya 4), Mulberry multivoltine (mmyc) X Mulberry bivoltine (China 2), Mulberry multivoltine(mmyc) x Mulberry bivoltine (Kenya 5), Mulberry multivoltine(mmyc) X Mulberry bivoltine (korea 1), Mulberry multivoltine yellow coccon, Mulberry bivoltine Kenya 4, Mulbery Biviltine Kenya 5, Mulbery Biviltine Korea 1, Mulbery Biviltine China 2). To conduct the activity, alcohol, table knife, Mulberry silkworm, thermo-hygro-meter, mountage, humidifier, sensitive balance, leaf chopper, leaf storage box, feeding tray, shelfing box, plastic sheet, razorblades and disinfectant chemicals were prepared. Parent bivoltines and their hybrids were treated with HCl chemicals to break the diapousing mechanisms. The existing mulberry multivoltine (yellow coccon), Kenya and Korea bivoltine silkworms were used for this study because the races are having different characteristic features. Mulberry multivoltine (yellow coccon color) has relatively disease resistant ability than bivoltine but the coccon size is very small. Mulberry feeding Kenyan's and korean's bivoltine have big cocoon size, however, diseases tolerant capability were very low. Therefore, higher disease tolerant ability and yellow coccon color, and lower disease tolerant ability and big coccon sizes are significant characteristic features of mulberry multivoltine and bivoltine silkworms, respectively, used for experimentation. Prior to the implementation of the hybridization, isolated room was prepared for hybridization of the silkworm races to avoid contact with the other species. In the isolated room, different boxes of the same sizes, which are having sufficient air circulation system,were prepared for matting of the adult races and growing of larvae is until mounting. An equal age and sexually matured mulberry multivoltine yellow as Female (PA1) and mulberry feeding Korea, China and Kenyans(K4 and K5) bivoltines as a male (PB1, PB2 and PB3) were used for the first phase combination/ hybridization (PA1 x PB1= F1-1, PA1 x PB2=F1-2, PA1 x PB3=F1-3 and PA1 x PB4=F1-4 hybrid). For each parent A and B, 10 females and male were taken and mating was performed in the mating boxes. Four hybrids were produced (F1-1, F1-2, F1-3, and F1-4) and mating was performed in the mating boxes and stayed for about 5-6 hrs in the mating room. After 5-6 hours of mating, male parents were discarded and female was put in the egg laying boxes. An egg laying required 18-24 hours depending on environmental conditions of the experimental room. After 18-24hrs of an egg laying, an eggs was put in the egg hatching boxes. Hatching of an egg was performed from 10^{th} to 14^{th} day's oviposition. When an egg was hatched 90% and above, 200 newly arrived, young larvae's were transferred to feeding boxes. In each instars' of larval durations in the feeding boxes, carful observation and recording of all the necessary data were performed on the hybrids as well as parents, up to seventh generations. The experiment was repeated for four times and the genetic expression of the hybrids on different economic traits [Pupation rate (%),number of diseased larvae, fecundity, survival rate (%), cocoon weight (g), cocoon shell weight (g), cocoon shell ratio (%) and filament length (m)] were recorded and compared with original parents.

RESULTS AND DISCUSSIONS

The silkworm breeding is the most important example where heterosis is being exploited commercially to the maximum extent. To achieve desired goals, cross breeding is widely used in commercial animal production as a means of exploiting heterosis (Sang, 1956 and Bowman, 1959). Significant differences were observed for all economic parameters among different hybrids of polyvoltinex bivoltine crossing as well as parents. Hatchability with chemical significantly higher in hybrids and mulberry mutivotine followed by parent's bivltines. After the eggs are laid, if they are subjected to an artificial treatment at appropriate embryonic developmental stage, it is possible to stimulate further growth of the embryo (Thalaghattapura, 2005). But hatchability without chemical was nil for all bivoltine parents, however, significantly higher for multivoltine and hybrids (Table 1- Table 7). This is because once the embryo enters diapause, it hibernates through a specific period of time and when it experiences favourable temperature, it resumes its growth and then hatches (Roy *et.al.*, 1997). Roy *et. al.*, (1997) and Hua Wang et. al., (2015) also explained that, in order to get maximum yield of silk from the silkworms, appropriate environmental conditions were required for both multivoltine and bivoltine silkworms. Larval period significantly lower in mulberry multivoltine followed by mulberry bivoltine china2 and hybrid (china2 x mmyc), however, significantly higher larval period were observed in the other treatments (Table 1). This is in agreement with Basavarag (2005) reported that, naturally, mulberry multivoltine silkworms have lower larval period and relatively higher diseases resistant but lower cocoon yield compared to bivotines silkworms. Self-crossing of F1 generations of multivoltine x bivoltines hybrids were performed and evaluated for F2, F3 and F4 generations (Table 2). Insignificant and similar results were observed for number of eggs laid by adults and cocooning percentage but significant results were recorded for others parameters. Larval weight, larval period and filament length considerably higher in parent bivoltines followed by hybrids but very low in parent multivoltine (Table 2). This is in accordance with Basavaraja (2005) and Hua Wang et. al., (2015) reported that, the hybrid vigor should not be expressed in some generations of silkworm hybrids compared to parents due to lower gen manifestation for some hybrids of silkworms and environmental factors. Similarly, Basavaraja, 2005 also opined that, multivoltine silkworms have lower cocconsize and short life period than bivoltine silkworms.

Numbers of eggs produce by adults, larva weight, cocooning percentage, filament length of hybrids and parents were uniform and significantly higher results were observed in F5,F6 and F7 generations (Tabe 3 and Table 4). According to Moorthy *et, al.* (2012), different results were observed, the pupation (%) ranged between 75.0 and 90.12 in the bi x multi hybrids as compared to 8.84 & 36.0% in the bivoltine breeds. Nearly two to nine fold increase of pupation % was noted in the Bi x Multi hybrids than conventional bivoltine, this is probably due to silk worm race and environmental variations of experimental area. Silk ratios and survival rates significantly higher in hybrids than parents (Table 3 and Table 4). Our study was similar to the results of different authors' on the same area. Use of bi x multi hybrids in commercial seasons was studied by Roy *et al.*(1997) and Rao *et al.* (2006) the yopined that bi x multi hybrids performed better in both favorable and adverse season due to heterozygous superiority. It is also observed that, both bi x multi hybrids and multi x (bi x multi) hybrids performed better during adverse seed crop season and adverse commercial season respectively. In addition, the superiority of the hybrids over parental strains is undoubtedly due to variable magnitude of heterosis for the quantitative characters in silkworm and the results of present study are corroborating the findings of Gamo (1976). On the other hands, hybrid vigor is an important tool in increasing cocoon production, evaluation, maintenance of inbredlines and identification of promising hybrids for commercial exploitation (Nagaraju et.al., 1996 and Hosne et. al.,2014). Besides, they opined that bi x multi hybrids performed better in adverse season due to heterozygous superiority. The superiority of the hybrids over parental strains is undoubtedly due to variable magnitude of heterosis for the quantitative characters in silkworm and the results of present study are corroborating the findings of Gamo (1976).

Larval period significantly higher in parents than hybrids for all generations (F5- F7) Table 3 and Table 4. According to Moorthy *et, al.* 2012, the larval period of bivoltine x multivoltine hybrids was less (1.5 days to 3.0 days) as compared to the bivoltine parents. Similarly, Larval period was less in bi x multi hybrids compared to conventional bivoltine breed for two reasons; one is because of heterogeneity and another is of male parent (Roy et, al. 1997). When a multivoltine, which possesses the shortest larval duration, is used as female component of the cross (bi x multi), the F1 larvae matures early, It is due to the fact that male component of the cross-carrying maturity gene, i.e., early maturity in multivoltine and late maturity in bivoltine on the Z chromosomes influences the larval duration (Tazima,1964).

Table 1. Evaluation of **F1** generation of polyvoltine x bivoltines silkworm hybrids for some economic parameters.

No	Treatment	Fecundity (No)	Hatchability With chemical (%)	Hatchability Without chemical (%)	Average of 10 larval weight (g)	Larval period (days)	Survival rate (%)	Cocooning percentage	Silk ratio (%)	Filament length (m)
1	**Myc x Kenya4**	333.69a ± 6.33	81.90c ± 0.89	60.33b ± 2.60	36.91b±0.91	26.62a ± 0.20	76.09 c ±1.23	97.78ba±0.73	19.82ed ± 0.42	708.12b±5.58
2	**Myc x Kenya5**	341.18a ±4.97	83.71bc ± 1.57	60.67b ±1.76	37.11b±0.38	26.66a ± 0.33	78..02cb±0.85	97.65ba±0.46	20.40ecd±0.32	722.42b±5.78
3	**Myc x China2**	377.28a ±16.66	86.51b ± 0.67	60.66b ± 2.33	38.02b± 2.13	25.51bc ± 0.24	78.13cb±0.77	97.32ba±0.58	22.53ba ±1.09	721.73b±12.90
4	**Myc x Korea1**	335.24a ±7.11	81.91c ± 1.33	62.65b ± 3.00	36.54b±0.06	26.66a ± 0.33	78.09cb±1.09	97.63ba±0.67	19.63e ±0.35	728.26b±19.59
5	**Myc(multivoltine)**	340.34a ±17.51	96.31a ± 1.14	97.06a ± 0.47	**32.43c**±0.41	23.33d ±0.33	91.97a ±0.32	97.72ba±0.43	23.86a ±0.90	575.88c±19.07
6	**China2 (bivoltine)**	335.93a± 14.77	85.24bc± 0.62	00.00c ± 0.00	42.06a±1.01	25.00c ±0.57	80.97b ±1.14	98.16a ±0.95	21.96bc±0.23	793.18a±15.23
7	**Kenya4(bivoltine)**	352.19a± 25.91	83.93bc± 0.85	00.00c ± 0.00	43.87a±1.01	26.40ba±0.30	76.43c ±1.28	96.65ba±0.00	19.30e ±0.05	786.67a±9.22
8	**Kenya5(bivoltine)**	349.01a± 19.54	84.12bc± 1.31	00.00c ± 0.00	42.46a±0.72	26.66a ±0.33	74.76c ±3.05	97.44ba±0.00	20.14ed±0.63	769.73a±8.13
9	**Korea1(bivoltine)**	329.78a± 10.88	82.67c ± 1.23	00.00c ± 0.00	43.69a±0.75	26.66a ±0.33	76.19c ±1.20	66.23b ±0.31	21.53bcd±0.45	771.84a±9.65

Table 2. Evaluation of average data of F2, F3 and F4 generations of multivoltine x bivoltine silkwormh ybrids for some economic parameters.

No	Treatment	Fecundity (No)	Hatchability With chemical (%)	Hatchability Without chemical (%)	Average of 10 larval weight (g)	Larval period (days)	Survival rate (%)	Cocooning percentage	Silk ratio (%)	Filament length (m)
1	**Myc x Kenya4**	382.40a+18.72	94.05a+1.90	50.00b+0.00	38.04b+0.24	23.07dc+0.07	88.58b+1.06	97.25a+0.87	22.67a+0.24	737.57b+11.88
2	**Myc x Kenya5**	384.32a+24.19	93.49a+0.26	50.00b+2.88	36.74b+0.36	23.00dc+0.00	87.20b+0.13	97.47a+0.67	23.15a+0.69	731.25b+4.00
3	**Myc x China2**	**357.25a**+22.82	93.56a+1.50	48.33b+1.66	38.67b+0.85	23.00dc+0.00	89.92ba+0.73	97.08a+0.23	22.39a+0.18	736.55b+7.90
4	**Myc x Korea1**	375.42a+5.60	93.88a+1.53	48.00b+3.00	37.80b+0.74	23.33c+0.33	86.79b+0.36	96.78a+0.35	22.13a+0.38	737.23b+8.74

Table 2. Continuation

5	**Myc (multivoltine)**	380.92a+22.78	96.66a+0.42	96.67a+0.33	**31.52c**+0.69	22.66d+0.33	**92.76a**+0.43	96.90a+0.34	22.68a+0.56	592.74c+14.64
6	**China2 (bivoltine)**	399.01a+13.72	85.74b+0.31	0.00c+0.00	41.51a+0.75	26.70a+0.20	81.69c+1.79	96.78a+0.35	22.15a+0.64	769.12a+8.65
7	**Kenya4(bivoltine)**	385.68a+23.36	84.45b+1.09	0.00c+0.00	41.86a+0.75	26.31a+0.03	75.52d+2.88	97.18a+0.26	19.25b+0.43	784.22a+8.69
8	**Kenya5(bivoltine)**	383.91a+7.99	86.52b+ 3.48	0.00c+0.00	43.25a+0.54	25.57b+0.39	76.01d+1.52	96.84a+0.30	19.76b+0.35	767.44a+1.09
9	**Korea1(bivoltine)**	366.18a+0.63	85.65b+1.24	0.00c+0.00	41.89a+0.98	25.44b+0.29	76.29d+1.16	97.44a+0.63	20.14b+0.73	765.16a+3.35

Table 3. Evaluation of average data of F5 and F6 (generations) hybrids of polyvoltine xbivoltinesilkworm for some economic parameters.

No	Treatment	Fecundity (No)	Hatchability With chemical (%)	Hatchability Without chemical (%)	Average of 10 larval weight (g)	Larval period (days)	Survival rate (%)	Cocooning percentage	Silk ratio (%)	Filament length (m)
1	**Myc x Kenya4**	354.52b±10.07	93.66ba±1.34	53.33b±1.66	42.08a±0.06	22.78dc±0.23	88.14bc±0.99	97.11a±0.43	21.60bac±0.12	765.31ba±4.33
2	**Myc x Kenya5**	375.26a±13.48	92.61b±0.29	53.66b±1.85	40.75a±0.46	23.07c±0.06	87.66bc±1.27	97.18a±0.72	22.72ba±0.34	763.16ba±11.88
3	**Myc x China2**	372.32ba±12.25	95.40a±1.59	53.33b±1.66	40.93a±1.26	23.00c±0.00	89.91ba±0.56	96.24a±0.55	22.82a±0.24	763.51ba±4.15
4	**Myc x Korea1**	385.96a±18.10	93.86ba±0.33	55.67b±0.33	40.44a±0.50	22.80dc±0.41	85.73c±0.62	96.98a±0.37	22.71ba±0.51	745.01b±0.24
5	**Myc (multivoltine)**	371.96ba±16.01	95.39a±0.67	96.00a±0.00	29.76b±2.77	22.00d±0.01	92.12a±1.18	97.08a±0.23	23.23a±0.12	493.56c±17.95
6	**China2 (bivoltine)**	380.99a±22.89	86.57c±0.58	00.00c±0.00	41.84a±0.31	26.00a±0.00	80.41d±1.86	96.31a±0.63	19.97c±1.22	763.39ba±3.47
7	**Kenya4(bivoltine)**	385.25a±13.92	83.19d±0.56	00.00c±0.00	41.80a±0.90	25.17ba±0.63	77.05ed±1.63	96.00a±0.38	21.18bac±0.45	765.58ba±10.34
8	**Kenya5(bivoltine)**	373.44ba±20.92	83.84d±0.90	00.00c±0.00	39.73a±0.19	25.07ba±0.63	77.87ed±0.67	96.55a±0.30	19.86c±0.50	762.25ba±3.00
9	**Korea1(bivoltine)**	386.18a±29.16	79.74e±0.20	00.00c±0.00	41.24a±0.62	24.73b±0.37	74.34e±1.28	95.85a±0.80	20.73bc±1.16	775.78a±14.19

Table 4. Evaluation of **F7** generation hybrids of polyvoltine xbivoltines silkworm for some economic parameters.

No	Treatment	Fecundity (No)	Hatchability With chemical (%)	Hatchability Without chemical (%)	Average of 10 larval weight (g)	Larval period (days)	Survival rate (%)	Cocooning percentage	Silk ratio (%)	Filament length (m)
1	**Myc x Kenya4**	362.00a±3.65	93.75ba±1.45	41.67b± 4.40	44.74a±1.50	22.23b±0.05	86.78b±0.39	96.60ba±0.32	23.06ba±0.80	757.75a±16.3
2	**Myc x Kenya5**	383.26b±14.37	93.51ba±1.30	45.00b±2.88	44.34a±1.20	21.09c±0.07	88.97ba±1.21	97.45a±0.56	22.41ba±0.37	764.62a±10.4
3	**Myc x China2**	400.22ba±7.26	94.59ba±0.90	40.00b± 2.88	44.87a±0.70	21.53cb±0.39	87.72b±1.66	96.55ba±0.30	22.85ba±0.39	752.34a±6.64
4	**Myc x Korea1**	374.11b±13.74	91.78b±1.14	41.33b± 3.67	43.916b±0.33	21.28cb±0.56	86.77b±0.33	95.89b±0.32	22.34b±0.12	766.00a±9.77
5	**Myc (multivoltine)**	368.95b±22.78	96.71a±0.59	96.34a±0.33	**35.7**0c±0.32	21.93cb±0.37	92.93a±0.31	97.61a±0.33	**23.62a**±0.16	487.49b±18.7
6	**China2 (bivoltine)**	425.29a±12.97	83.19c± 1.52	00.00c± 0.00	44.71a±0.56	25.74a±0.25	80.33c±2.56	97.62a±0.41	**19.75c**±0.11	768.43a±9.14
7	**Kenya4(bivoltine)**	398.13ba±8.04	82.23c±1.01	00.00c±0.00	45.39a±0.14	25.34a±0.33	78.30c±1.12	96.65ba±0.40	19.71c±0.15	752.36a±6.50
8	**Kenya5(bivoltine)**	395.49ba±14.43	82.92c±0.61	00.00c±0.00	44.20a±0.82	25.45a±0.29	77.88c±1.68	96.68ba±0.43	20.63c±0.61	764.67a±0.77
9	**Korea1(bivoltine)**	399.08ba±8.08	83.30c± 2.03	00.00c±0.00	45.16a±0.33	25.77a±0.39	78.16c±1.06	96.56ba±0.78	19.74c±0.19	766.18a±15.5

CONCLUSION

Multivoltinex bivoltine mulberry silkworms crossing were performed and evaluated up to seventh generation (F7) by using some economic parameters. Hybrids of multivoltine x bivoltines silkworms are better than individual parents' in terms of relative disease resistant and quality of silk produced. Significantly (P<0.01) higher survival rates and silk ratios were observed at generation five (F5)and above than the other generations for maximum silk production. Their for it can be conclude that, fortha maximum and sustainable silk production from silkworms, F5 generation hybrids of multivoltine x bivoltines silkworms are better than individual parents for the end users.

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