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Efficacy of bio products on larval population of greater wax moth, (*Galleria mellonella*)

Lal Bahadur Singh, Ashok S Yadav and Vishnoo Omar

Abstract
A lab experiment was carried out to evaluate the effect of bio products on percent mortality of third and sixth instar *Galleria mellonella* larvae by the testing of different concentration *viz*. Pongamia oil and Citronella oil (at 0.3, 0.4 and 0.5% concentration), NSKE, Garlic clove extract and Tobacco leaf extract (at 3, 4 and 5% concentration) and *Bacillus thuringiensis* (Biobit and Biolep; at 5, 7 and 9% concentration) each tested at 1, 3, 5 and 7 days after treatment for the assess the effect of different concentrations of bio products on greater waxmoth. The experiment conducted on percent mortality or the lethality of all bio products was depends on concentration of the pesticides, higher concentration doses caused higher mortality percent in both third and sixth instar larval stage of greater wax moths. For the higher mortality percent was recorded at 7 days after treatment application on both third and sixth instar larval stage of greater wax moths. The entire management study shows Bt, Biobit (32,000 IU mg^{-1}) @ 9 gm concentration, Citronella oil @ 0.5% concentration and Pongamia oil @ 0.5% concentration can be recommended for the management of *G. mellonella* larva in bee hives.

Keywords: *Galleria mellonella*, bio products, bacillus thuringiensis, formulations, larvae, mortality

Introduction
Honeybees are highly valued resource insects around the world, prized not only for production of honey, wax and other products. The pests and diseases have severely reduced the number of healthy colonies available for beekeeping as well as the hive products harvested from the colonies (Surendra *et al*., 2010) [9]. Two species of wax moth known to be harmful to bee colonies and stored beeswax are greater wax moth (Lepidoptera: Pyralidae, *Galleria mellonella*) and lesser wax moth (Lepidoptera: Pyralidae, *Achroia grisella*). Both of these species have the same type of tunneling habits, but greater wax moth, *Galleria mellonella*, causes the greatest damage in apiaries. The larvae of both moths feed on wax, pollen and cocoons of the bee larvae (Ellis *et al*., 2013) [2]. Unless controlled at early stage, this leads to the destruction of honeycombs and subsequent deterioration of the weakened colonies and can cause significant damage to stored beekeeping equipment. Wax moth is widely distributed in Ethiopia and was reported the most troublesome pest of honeybees. According to (Almadani and Hiware, 2020) [1], wax moths cause loss of 2.5, 2.9 and 2.5 tons of pure beeswax in West Shoa, South West Shoa and East Shoa Zones, respectively.
Plant based pesticides have been adopted in different countries and cultures with their own specific indigenous knowledge and parallel standards and methods for evaluation. Available literature shows that volatile oils from Eugenia aromatic (Clove) and basil (*Ocimum basilicum*) were applied to control wax moth larvae and highly effective (Zaitoun, 2007) [14]. However, the safety, effectiveness and quality of botanical products depend on the quality of their source materials and how elements are handled through production processes.

Materials and Methods
Studies on growth and development of Greater waxmoth on different bio products were carried out in 2020-2022 at ZARS-KVK Morena, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior (MP). The experiment was conducted on larval activity of *G. mellonella*. The two different oils were mixed with water separately to obtain a concentration of 0.3, 0.4 and 0.5 percent. For making the plant extract, first crushed the material and then soaked in water for 24 hours. After 24 hours the liquid was strained through a fine muslin cloth and the additional quantity of water was added to make-up the volume for required concentrations of three, four and five percent. The laboratory cultured, third and sixth instar larvae of wax moth was selected and released separately at the rate of ten larvae for each treatment.

Observations were made once in 24 hours, to record larval mortality of greater wax moth for a period of seven days. Number of larvae died was expressed as percentages (Table 1).

The commercial formulations of *Bacillus thuringiensis* Var. *kurstaki viz*., Biobit (32,000 IU mg^{-l}) and Biolep (16,000 IU mg^{-l}) was tested against larvae of greater wax moth under laboratory conditions. These chemicals were mixed with water to obtain an emulsion of different dosages (5, 7 and 9 grams). The combs were dipped in these emulsions and dried under a fan. The laboratory cultured third instar and sixth instar larvae of greater wax moth was selected and released separately at the rate of ten larvae for each treatment. Each treatment was replicated thrice. Observations were recorded once in 24 hours on mortality of greater wax moth larvae for a period of seven days (Table 1).

The data recorded on effect of different concentration of bio products on the third and sixth instar larvae of greater waxmoth at 1 day, 3 days, 5 days and 7 days after treatment.

Result

A trial was laid out under laboratory conditions to evaluate the efficacy of the bio products *viz*., Pongamia oil, Citronella oil, NSKE, Garlic clove extract, Tobacco leaf extract and *Bacillus thuringiensis* (Biobit and Biolep) against the larvae of Greater waxmoth. An equal number of larvae were released for all the treatments and concentrations. The larval mortality was recorded at different days after treatment. The results of the trial are presented in the following pages.

Effect of bio products against third instar larvae of Greater waxmoth

At 24 hours (1 day) after the treatment there is no significant effects showed by the all treatments but maximum mortality percent was recorded in Pongamia oil @ 0.5% concentration (46.32%) followed by Tobbaco leaf extract @ 5% concentration (40.23%) which was at par with *Bt.*, Biobit (32,000 IU mg^{-l}) @ 9 gm concentration (40.03%) and Citronella oil @ 0.5% concentration (33.79%) .

After 3 days of treatments application showed significant effect on larval mortality percent, maximum mortality percent was recorded in *Bt*., Biobit (32,000 IU mg^{-l}) @ 9 gm concentration (88.89%) followed by Citronella oil @ 0.5% concentration (74.78%), *Bt*., Biobit (32,000 IU mg^{-l}) @ 7 gm concentration (71.12%), Citronella oil @ 0.4% concentration (67.56%) and Tobbaco leaf extract @ 5% concentration (59.45%) while, minimum mortality percent was recorded in Pongamia oil @ 0.3% concentration (03.78%) followed by Garlic clove extract @ 3% concentration (06.67%), Tobbaco leaf extract @ 3% concentration (08.89%) and Bt., Biolep (16,000 IU mg-1) @ 5 gm concentration (11.11%). Whereas, no mortality was found in treatment NSKE @ 3% concentration and Untreated check (Tab. 2 & Fig. 1).

At 5 days of treatments application showed significant effect on third instar larval percent mortality, highest percent mortality was recorded in *Bt*., Biobit (32,000 IU mg^{l}) @ 9 gm concentration (92.67%) followed by Citronella oil @ 0.5% concentration (89.68%), Citronella oil @ 0.4% concentration (83.66%), Pongamia oil @ 0.5% concentration (78.78%) and Tobbaco leaf extract @ 5% concentration (70.00%) while, minimum mortality percent was recorded in NSKE @ 4% concentration and Pongamia oil @ 0.3% concentration (each 13.33%, respectively) followed by Tobbaco leaf extract @ 3% concentration (14.33%) and *Bt.*, Biolep (16,000 IU mg^{-l}) @ 5 gm concentration (20.00%). Whereas, no mortality was found in treatment NSKE @ 3% concentration and Untreated check (Tab. 2 & Fig. 1).

At 7 days after treatment application showed significant effect on larval mortality percent, maximum mortality percent was recorded in *Bt*., Biobit (32,000 IU mg^{l}) @ 9 gm concentration (93.00%) followed by Citronella oil @ 0.5% concentration (90.89%), Pongamia oil @ 0.5% concentration (87.55%), Citronella oil @ 0.4% concentration (85.33%) and *Bt*., Biobit (32,000 IU mg^{-l}) @ 7 gm concentration (81.44%) while, minimum mortality percent was recorded in Pongamia oil @ 0.3% concentration (13.33%) followed by NSKE @ 4% concentration (22.00%) and Tobbaco leaf extract @ 3% concentration (24.00%). Whereas, no mortality was found in treatment NSKE @ 3% concentration and Untreated check (Tab. 2 & Fig. 1).

Effect of bio products against sixth instar larvae of Greater waxmoth

At one day after treatment application there is no significant effects showed by the all treatments but maximum mortality percent was recorded in Garlic clove extract @ 5% concentration (21.00%) followed by Citronella oil @ 0.5% concentration (14.77%) and Tobacco leaf extract @ 5% concentration (13.55%).

After 3 days of treatments application showed significant effect on larval mortality percent, maximum mortality percent was recorded in Citronella oil @ 0.5% concentration (43.01%) followed by Pongamia oil @ 0.5% concentration (39.34%), Garlic clove extract @ 5% concentration (37.77%), *Bt*., Biobit (32,000 IU mg^{-l}) @ 9 gm concentration (35.77%) and Tobacco leaf extract @ 5% concentration (32.34%) and it was at par with Citronella oil @ 0.4% concentration (32.22%) while, minimum mortality percent was recorded in Garlic clove extract @ 3% concentration (02.22%) followed by *Bt*., Biobit (32,000 IU mg^{-l}) @ 5 gm concentration (09.55%) and Tobbaco leaf extract @ 3% concentration (10.00%). Whereas, no mortality was found in treatment Pongamia oil @ 0.3% concentration, NSKE @ 3% concentration, NSKE @ 4% concentration and Untreated check (Tab. 3 & Fig. 2).

At 5 days of treatments application showed significant effect on sixth instar larval mortality percent, maximum mortality percent was recorded in *Bt*., Biobit (32,000 IU mg^{l}) @ 9 gm concentration (64.24%) followed by Citronella oil @ 0.5% concentration (63.33%), Garlic clove extract @ 5% concentration (62.34%), Pongamia oil @ 0.5% concentration (61.23%) and Tobbaco leaf extract @ 5% concentration (58.89%) while, minimum mortality percent was recorded in Garlic clove extract @ 3% concentration (08.23%) followed by Tobacco leaf extract @ 3% concentration (15.55%) and *Bt*., Biolep (16,000 IU mg^{-l}) @ 5 gm concentration (20.00%). Whereas, no mortality was found in treatment NSKE @ 3% concentration, NSKE @ 4% concentration and Untreated check (Tab. 3 & Fig. 2).

At 7 days after treatment application showed significant effect on larval mortality percent, maximum mortality percent was recorded in *Bt*., Biobit (32,000 IU mg^{l}) @ 9 gm concentration (98.89%) followed by Garlic clove extract @ 5% concentration (78.22%), Tobacco leaf extract @ 5% concentration (76.45%), Pongamia oil @ 0.5% concentration (71.17%) and Citronella oil @ 0.5% concentration (66.66%) while, minimum mortality percent was recorded in Garlic clove extract @ 3% concentration (14.22%) and, it was at par with Tobacco leaf extract @ 3% concentration (14.73%)

followed by *Bt*., Biolep (16,000 IU mg^{-1}) @ 5 gm concentration (21.11%). Whereas, no mortality was found in treatment NSKE @ 3% concentration, NSKE @ 4% concentration and Untreated check (Tab. 3 & Fig. 2).

Table 1: Treatments details of different concentrations of bio products.

Treatment code	Treatment name	Percent concentration	Treatment code	Treatment name	Percent concentration
T1	Pongamia oil	0.3	T12	GCE	5
T2		0.4	T13	Tobacco leaf extract	3
T3		0.5	T14		4
T4	Citronella oil	0.3	T15		5
T5		0.4	T16	Bt, Biobit (32,000 IU mg^{-1})	5
T6		0.5	T17		7
T7	NSKE	3	T18		9
T8		4	T19	Bt, Biolep (16,000 IU mg^{-1})	5
T9		5	T20		7
T10	Garlic clove extract	3	T21		9
T11		4	T22	Untreated control	

(NSKE- Neem seed karnal extract, Bt- *Bacillus thuringiensis* Var. *kurstaki*)

Table 2: Efficacy of different bio products on growth and development of third instar larvae of *Galleria mellonella.*

3 Instar						
Treatment	**Bio-products**	**1 Day**	**3 Days**	**5 Days**	**7 Days**	**Mean**
T1	Pongamia oil @ 0.3% concentration	00.00(00.00)	03.78(06.55)	13.33(21.32)	13.33(21.27)	07.61(12.28)
T2	Pongamia oil @ 0.4% concentration	21.70(27.73)	21.11(27.36)	34.79(36.09)	65.55(54.04)	35.79(36.305)
T3	Pongamia oil @ 0.5% concentration	46.32(48.68)	58.66(55.35)	78.78(70.49)	87.55(81.26)	67.58(63.95)
T4	Citronella oil @ 0.3% concentration	00.00(00.00)	19.55(26.24)	35.33(36.41)	48.66(44.23)	25.89(26.72)
T5	Citronella oil @ 0.4% concentration	14.88(22.55)	67.56(55.29)	83.66(66.32)	85.33(67.48)	62.86(52.91)
T6	Citronella oil @ 0.5% concentration	33.79(35.50)	74.78(59.92)	89.68(71.28)	90.89(85.11)	74.28(62.95)
T7	NSKE @ 3% concentration	00.00(00.00)	00.00(00.00)	00.00(00.00)	00.00(00.00)	00.00(00.00)
T8	NSKE @ 4% concentration	14.89(22.66)	16.00(23.30)	13.33(21.39)	22.00(27.62)	16.55(23.74)
T9	NSKE @ 5% concentration	22.10(28.00)	19.11(25.93)	42.56(40.71)	74.33(59.69)	39.53(38.58)
T10	Garlic clove extract @ 3% concentration	06.45(14.67)	06.67(12.29)	22.56(28.29)	38.67(38.44)	18.59(23.43)
T11	Garlic clove extract @ 4% concentration	07.99(16.44)	14.44(22.30)	30.00(33.19)	62.56(52.26)	28.75(31.05)
T12	Garlic clove extract @ 5% concentration	10.66(19.05)	42.12(40.43)	62.22(52.11)	72.56(58.61)	46.89(42.55)
T13	Tobbaco leaf extract @ 3% concentration	00.00(00.00)	08.89(14.26)	14.33(22.23)	24.00(29.29)	11.80(16.45)
T14	Tobbaco leaf extract @ 4% concentration	19.11(25.89)	28.66(32.34)	26.66(31.11)	55.55(48.21)	32.50(34.39)
T15	Tobbaco leaf extract @ 5% concentration	40.23(39.34)	59.45(50.44)	70.00(56.81)	74.33(66.36)	61.00(53.24)
T16	Bt, Biobit (32,000 IU mg^{-1}) @ 5 gm concentration	00.00(00.00)	56.01(60.67)	66.01(70.64)	76.00(71.56)	63.75(50.72)
T17	Bt, Biobit (32,000 IU mg^{-1}) @ 7 gm concentration	21.23(27.41)	71.12(68.23)	77.34(71.89)	81.44(76.87)	73.03(61.10)
T18	Bt, Biobit (32,000 IU mg^{-1}) @ 9 gm concentration	40.03(50.79)	88.89(76.99)	92.67(88.09)	93.00(90.00)	78.65(76.47)
T19	Bt, Biolep (16,000 IU mg^{-1}) @ 5 gm concentration	02.88(06.2)	11.11(19.42)	20.00(26.56)	23.01(28.63)	14.25(20.20)
T20	Bt, Biolep (16,000 IU mg^{-1}) @ 7 gm concentration	05.00(10.30)	21.45(27.57)	23.55(29.02)	30.44(33.46)	20.11(25.09)
T21	Bt, Biolep (16,000 IU mg^{-1}) @ 9 gm concentration	05.22(16.54)	29.11(32.63)	47.77(43.74)	62.11(52.05)	36.05(36.24)
T22	Untreated control	00.00(00.00)	00.00(00.00)	00.00(00.00)	00.00(00.00)	00.00(00.00)
Mean		15.59(18.71)	34.93(33.52)	45.34(41.71)	56.06(49.38)	37.98(35.83)
Sem		1.20	2.65	1.29	2.57	7.92
CD (5%)		3.42	7.58	3.69	7.33	22.62

Table 3: Efficacy of different bio products on growth and development of six instar larvae *Galleria mellonella.*

6 Instar						
Treatment	**Bio-products**	**1 Day**	**3 Days**	**5 Days**	**7 Days**	**Mean**
T1	Pongamia oil @ 0.3% concentration	00.00(00.00)	00.00(00.00)	22.56(28.33)	35.36(36.49)	14.48(16.21)
T2	Pongamia oil @ 0.4% concentration	00.00(00.00)	29.11(32.63)	45.55(42.46)	64.66(54.75)	36.46(33.16)
T3	Pongamia oil @ 0.5% concentration	12.22(20.41)	39.34(38.83)	61.23(51.52)	71.17(57.53)	44.86(41.38)
T4	Citronella oil @ 0.3% concentration	00.00(00.00)	18.66(25.49)	31.11(33.86)	56.56(48.78)	26.58(27.03)
T5	Citronella oil @ 0.4% concentration	08.22(16.10)	32.22(34.56)	56.66(48.84)	62.14(52.02)	39.81(37.88)
T6	Citronella oil @ 0.5% concentration	14.77(22.54)	43.01(40.97)	63.33(52.71)	66.66(54.75)	46.94(42.74)
T7	NSKE @ 3% concentration	00.00(00.00)	00.00(00.00)	00.00(00.00)	00.00(00.00)	00.00(00.00)
T8	NSKE @ 4% concentration	00.00(00.00)	00.00(00.00)	00.00(00.00)	00.00(00.00)	00.00(00.00)
T9	NSKE @ 5% concentration	10.79(19.10)	13.12(21.14)	25.55(30.36)	26.00(30.64)	18.86(25.31)
T10	Garlic clove extract @ 3% concentration	00.00(00.00)	02.22(06.98)	08.23(16.62)	14.22(22.12)	06.17(11.43)
T11	Garlic clove extract @ 4% concentration	12.77(20.75)	21.11(27.28)	31.11(33.89)	64.34(53.33)	32.33(33.81)
T12	Garlic clove extract @ 5% concentration	21.00(27.26)	37.77(37.86)	62.34(52.16)	78.22(62.22)	49.83(44.88)
T13	Tobbaco leaf extract @ 3% concentration	00.00(00.00)	10.00(15.17)	15.55(23.21)	14.73(22.55)	10.07(15.23)
T14	Tobbaco leaf extract @ 4% concentration	11.00(19.33)	23.33(28.86)	24.33(29.52)	43.33(41.16)	25.50(29.72)
T15	Tobbaco leaf extract @ 5% concentration	13.55(21.61)	32.34(34.61)	58.89(50.13)	76.45(60.98)	45.31(41.83)
T16	Bt, Biobit (32,000 IU mg^{-1}) @ 5 gm concentration	07.00(15.32)	09.55(18.01)	23.22(28.77)	42.14(40.47)	20.48(25.65)

T17	Bt, Biobit (32,000 IU mg^{-1}) @ 7 gm concentration	08.01(16.40)	12.56(20.72)	27.44(31.60)	63.23(52.69)	27.81(30.35)
T18	Bt, Biobit (32,000 IU mg^{-1}) @ 9 gm concentration	07.15(15.43)	35.77(36.72)	64.24(53.32)	98.89(86.51)	51.50(47.99)
T19	Bt, Biolep (16,000 IU mg^{-1}) @ 5 gm concentration	03.82(11.23)	09.12(17.45)	20.00(26.56)	21.11(27.33)	13.51(20.64)
T20	Bt, Biolep (16,000 IU mg^{-1}) @ 7 gm concentration	05.60(13.68)	17.78(24.84)	25.55(30.36)	28.56(32.30)	19.37(25.29)
T21	Bt, Biolep (16,000 IU mg^{-1}) @ 9 gm concentration	07.15(15.52)	27.77(31.81)	43.33(41.16)	57.45(49.28)	33.93(34.44)
T22	Untreated control	00.00(00.00)	00.00(00.00)	00.00(00.00)	00.00(00.00)	00.00(00.00)
Mean		06.50 (11.58)	18.85 (22.45)	32.28 (32.06)	44.87 (40.27)	25.63 (26.59)
Sem		1.02	2.15	1.05	1.10	7.39
CD (5%)		2.91	6.15	3.01	3.15	21.10

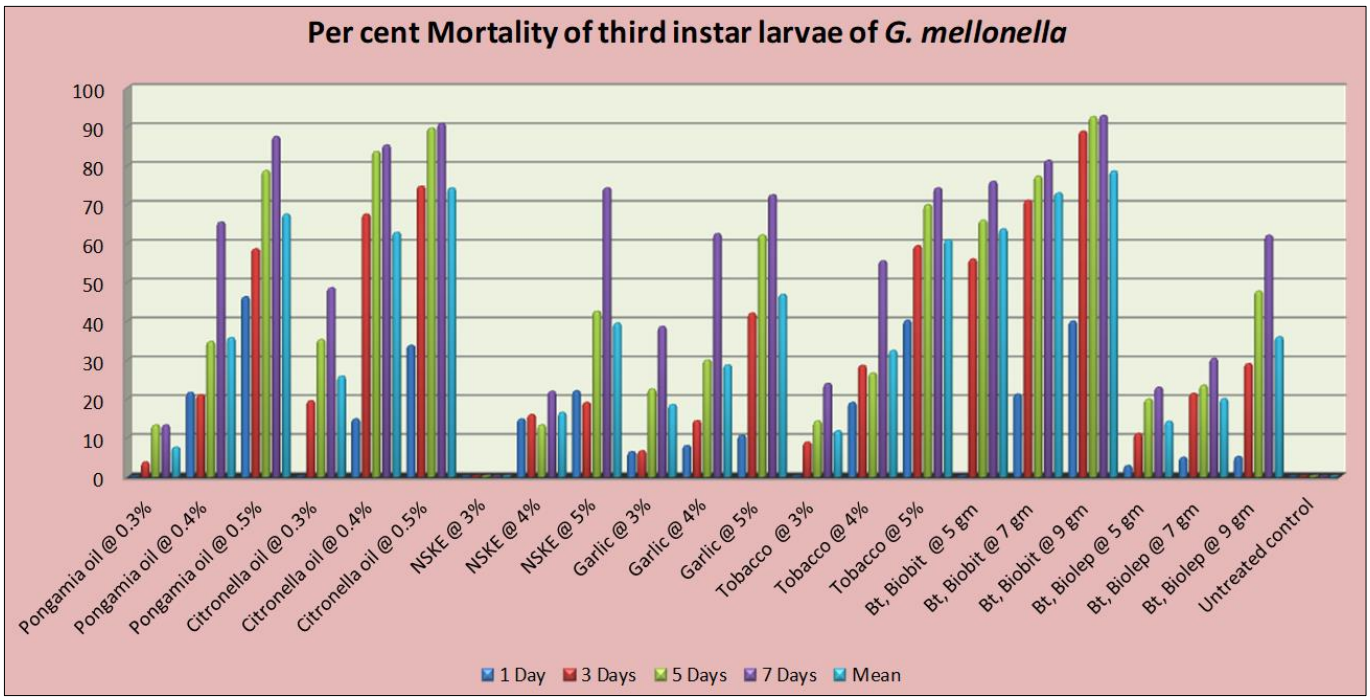

Fig 1: Percent Mortality of third instar larvae of *G. mellonella* at 1, 3, 5 and 7 days after treatment.

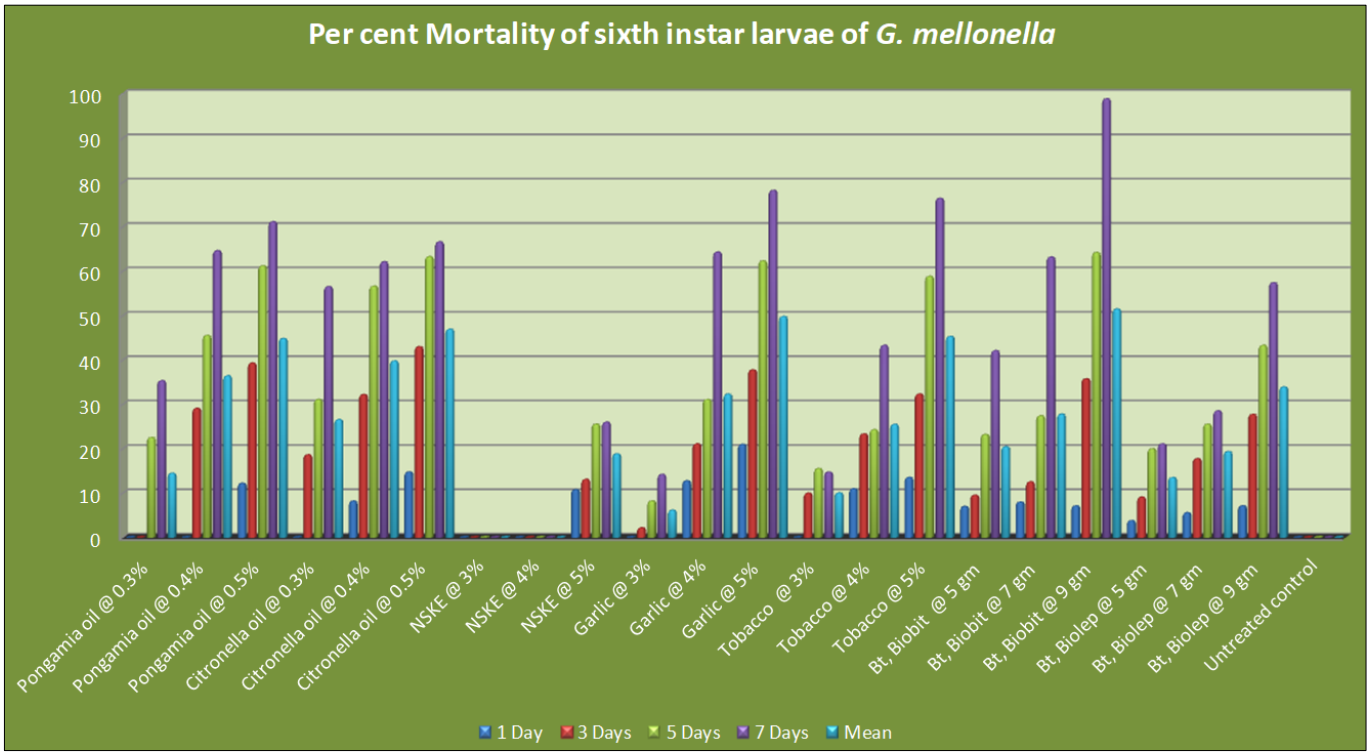

Fig 2: Percent Mortality of sixth instar larvae of *G. mellonella* at 1, 3, 5 and 7 days after treatment.

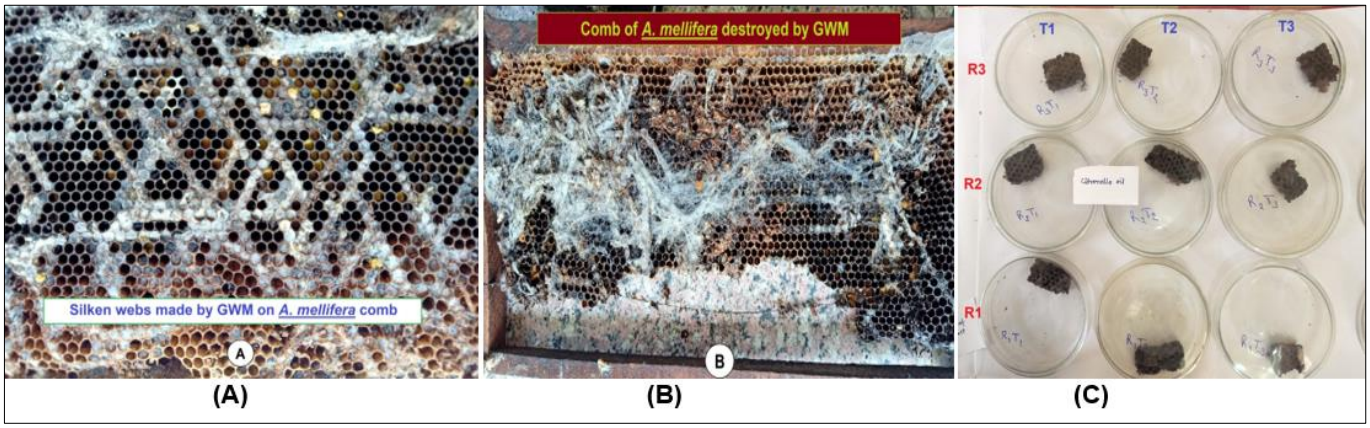

Fig 3: (A, B & C): *G. mellonella* larval infestation as making Silken Webs on *A. mellifera* colonies and lab work for efficacy of different bio products on larval population.

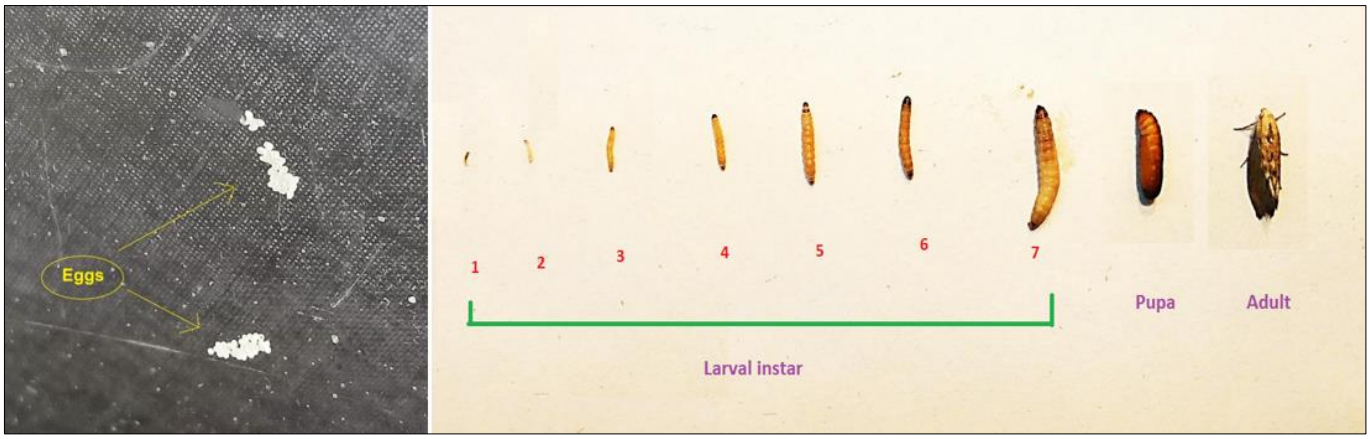

Fig 4: Growth and Development (Eggs, Larva, Pupa and Adult) of *G. mellonella* on *A. mellifera* colonies

Discussion

The comparison among treatments revealed that a highly significant difference was observed among the treatments even though all the bio products performed superior to the untreated check. The results on third instar larvae among the different concentrations of plant products, showed highest reduction in larval population in case of Citronella oil (0.5%) followed by Pongamia oil (0.5%) while, lowest mortality was observed in case of Pongamia oil (0.3%). Similarly, highest reduction in larval population among the different concentrations of pathogen, *B. thuringiensis* in case of *Bt*., Biobit (9, 7 and 5 gm) followed by *Bt*., Biolep (9 gm) while, lowest mortality was observed in case of *Bt*., Biolep (5 gm). The present finding supports from the findings of Telles *et al.* (2020) [11], Swamy *et al.* (2008) [10] and Viraktamath *et al.* (2005) [13], who reported that small larvae of greater wax moth were more susceptible to *Bt*. than the older larvae. Mahmoud and Abdul (2021) [4], Verma (1995) [12] and Swamy *et al.* (2008) [10] also studied the effect of biopesticides against *G. mellonella* larvae and found effective to controlling *G. mellonella* without any adverse effect on the honey bees. Swamy *et al.* (2008) [10] reported that the higher concentrations of plant products were most effective on percent mortality then lower concentration.

Similarly, on sixth instar larvae, among the different concentrations of plant products showed highest reduction in larval population in case of Garlic clove extract (5%) followed by Citronella oil (0.5%), while lowest mortality was observed in case of Garlic clove extract followed by Tobacco leaf extract (each 3%). Similarly, among *B. thuringiensis* products showed highest larval mortality in case of *Bt*., Biobit (9 gm) followed by *Bt*., Biolep (9 gm), while lowest larval mortality was observed in case of *Bt*., Biolep (5 gm). More or less similar result was obtained by Mohamed *et al.* (2014) [6], Swamy (2008) [10] and Rahman *et al.* (2017) [8]. Telles *et al.* (2020), Mandal and Vishwakarma (2018) [5] and Molin *et al.* (1987) [7] also found adequate control of *G. mellonella* by using B 401 (a formulation of Bt spores and crystals) for a longer period. Kwadha (2017) [3] and Verma (1995) [12] also witnessed a similar efficacy of Dipel on *G. mellonella* larvae and prove the effectiveness of higher concentration of bio products.

Conclusion

From the entire experimental investigation, it can be concluded that among plant products Pngamia oil and Citronella oil (eatch at 0.5% concentration) was superior then other concentrations of treatment groups to cause larval mortality while, among pathogens, *B. thuringiensis* products *Bt*., biobit (32,000 IU mg^{I}) at 9 gm concentration was most effective to percent mortality of *G. mellonella* larvae. It was also observed that the higher concentration of bio products cause higher larval mortality then lower concentration.

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