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Effect of temperature ramping on the mortality of mango mealy bug, *Droschia mangiferae* under laboratory conditions

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Abstract
Effective alteration of temperature regimes is found prudent to check the insect pest menace as insects are poikilothermic. Effect of high (40-50 ^{0}C), moderate (14 ^{0}c and18 ^{0}C) and low temperature (-4-0 ^{0}C) regimes on the survivability of mango mealy bug, *Droschia mangiferae* was noted in laboratory condition. High temperature regime starts from 40 ^{0}C and low temperature from -4 ^{0}C respectively. Temperature of both high and low was subsequently increased for 2 ^{0}C for each step. Extent of sample insect mortality, in gross, was in consonance with the degree of elevated high temperature and also with the retention time. Even exposure of 10-20 minutes was sufficient to achieve 100% mortality at 48^0 and 50 ^{0}C. However, moderate temperature of 14 ^{0}C and 18 ^{0}C has very little visible effect on the morbidity of the mealy bug. When the sample was kept at low temperature of 4 ^{0}C and 0 ^{0}C, for 10 and 20 minutes respectively, 86.7% and 100% died.

Keywords: Mango mealy bug, ramping temperature, survivability

1. Introduction
Mango (Mangifera indica L.) is a major fruit crop cultivated in India with an annual production of about 12 million tons [32]. West Bengal is the one of the largest state in consideration of the production of mango in India [22]. The Gangetic plain of West Bengal offer a congenial environment for mango production [33]. Out of the all administrative district of West Bengal, Malda ranks first with an annual net production of about 196 metric tons [11].

However, insect pests are the major threat to underscore the mango production accounting for huge seasonal loss. Application of insecticides of different newer brands, though in practice in large scale but very often is less effective to check the pest menace [23].

Among all of the mango insect pests, mealy bug, *Droschia mangiferae* is one of the notorious and destructive pests rendering huge scale of fruit loss [3]. Mealy bug is one of the major pests of mango and ranked 2nd after hopper in causing crop injury. Extent of loss may extend up to 50% [17] in some occasional cases. In general, *D. mangiferae* is found to infest almost all mango cultivars. Incidence of the pest starts from December, gains gradual momentum and attains the peak incidence during the middle of April when it is numerically more abundant. Damage to plants is primarily manifested due to the continuous sucking of 'cell sap' from tender leaves, stem, inflorescence and even from the fruits. Severe infestation often leads to fruit drops [17]. Further the honey dew following the excretion of *D. mangiferae* provides a medium for rapid growth of black and sooty fungi which decolorizes the fruit and makes it non-marketable [1, 34, 19].

The response of insects to temperature is very imperative to predict possible geographic range of a species and to develop phenological models to forecast pest population dynamics and its periodicity. For pest surveillance programmes the study of insect pest periodicity is imperative [26]. Thus tolerance to temperature can be a benchmark to estimate the relative extermination probability, to predict life history 'evolution' and to anticipate pest outbreaks for a particular species [39]. To assess the range of insect 'fitness' to different temperature regimes, laboratory experiment is imperative [40].

Insects being poikilothermic, have limited ability to regulate their body temperature. Temperature is one of the major important abiotic factors that regulate both the physiological and behavioural profile of insect organism [20, 21]. Insects are known to live in a wide range of thermal climates, but there is very little variability in the maximum temperature (40–50 °C)

which they can survive [18]. Clarke [8] narrated this temperature change in engineering terms as 'step function' and 'ramp function'. Step function refers to a change from one temperature to another as rapidly as possible. Step-function explains how speedily an insect can react to a thermal challenge. Ramp-function is when a slower rate of change in temperature occurs. Ramp-function heat treatments tells, through examination of the response curve, what mechanisms may be concerned in thermal tolerance and point out whether the tolerance limits of the insect are extended in response to a ramp- than to a step-function [8]. In thermal assays, temperature is gradually ramped 'up' or 'down' until insects lose consciousness that provide better estimate of thermal maxima or minima compared to traditional methods [36, 28]. Each organism commonly has a best fitted 'optimal range' of temperature at which it manifests highest level of physiological activity. 'Thermal stress' is thus found reflective effect on insect physiology and development.

Gross effect of agro-climatic factors on the incidence and numerical abundance of mealy bug was studied by workers like Karar *et al.*, [17] and Sathe *et al.*, [1] Though temperature is one of the important agro-climatic factors and interrelated to other agro-climatic components, very little reference on the effect of ramping temperature on mango mealy bug were noted.

Pest surveillance programme under modern IPM practice concerns more about the cultural and biological control than the toxic insecticide input. It is a complex task to develop an effective thermal treatment that offer required quarantine security yet has minimum unpleasant effect on product quality. Systematic research to develop such a treatment protocol requires an understanding of the effects of heat on insect mortality and product quality. Observation from ramping effect on the activity of the insect pest from laboratory condition can be befitted with the natural condition. The best fitted temperature that is pertinent to natural condition and causes maximum stress to insect population can thus be pointed out. The pest population under stress condition is less active and accordingly the pest control strategy at that time can thus be taken. As the insect population is in consonance with the temperature, the trends of population fluctuation i.e., the maximum and minimum incidence of pest thus can be predicted from this experiment as at the stressed condition, the population of *D. mangiferae* is supposed to be least and accordingly the cost of prophylactive measure for pest control would be decreased.

2. Materials and Methods

The Sample (Fig. 1a, b and c): Nymphs of early developmental instars of *D. mangiferae* during the period March-April, 2015 in three replications from orchard of the mango cultivar *Langra* were considered as the type specimen for the study.

Fig1: Mango mealy bug in its natural habitat (a) on stem (b) on leaf (c) on bark

3. Sample collection

Place of collection: Collection was mainly done from five adjacent mango orchards at Kaligram [25.38 ^{0}N-88.04 ^{0}E] that is located in the Chanchal-I block under the administrative jurisdiction of Malda District, West Bengal.

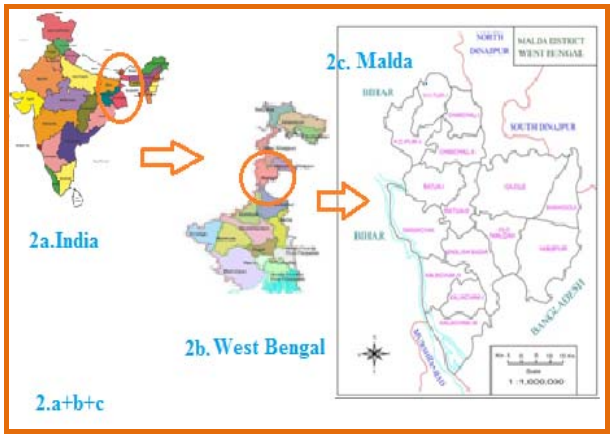

Fig 2. Map showing the sites of sample collection

Mode of collection: Twigs of mango branches were inspected at 2-day intervals during early morning and the samples were collected in sterilized glass vial having proper aeration with the help of fine camel brush. Sample was collected covering the calendar month of March-April during 2015.

4. Laboratory acclimatization

Fresh and tender mango leaves of the same cultivar were given to them as food. Food source was accordingly changes in alternative morning. The samples were kept for 24 hours at about 30 ^{0}-31 ^{0}C for acclimatization before the experiment. No food except water on need basis was given during the time of acclimatization.

5. Experimental design

5.1. The treatment: There were three temperature conditions and four temperature regimes **[a]** high (T1): 40-50 ^{0}C, **[b]** moderate: 14 ^{0}C (T2) and 18 ^{0}C (T3) and **[c]** low: -4 ^{0}C to 0 ^{0}C (T4). Specimens were kept at each temperature regimes for 10 minutes for T1, T2 and T3 treatment except for T4 where it was kept for 5 minutes.

5.2. The replication: For each experiment 30 mealy bug samples with three replications were used.

5.3. The instrument: (a) For hot treatment incubator of 250 liter open space capacity with model no.SCIN-3457146 and for **(b)** moderate and **(c)** cold treatment LG-refrigerator of 250 liter capacity with model no GL-195SADG5/2009 were respectively used. Freezer, chiller and freezer rack of the refrigerator have -4-0 ^{0}C, 14 ^{0}C and 18 ^{0}C temperature respectively. The temperatures of the respective part of the incubator and refrigerator were monitored by using digital thermometer.

5.4. Observations: Effect of different temperature regimes on the activity of *D. mangiferae* was recorded on the basis of the activity of the sample; Live (L) with normal movement, dead (D) with no movement, and knockdown (KD) with restricted movement. For this, after each treatment the insect samples were gently tingled by a needle and allowed for 5 minutes to show the response.

5.5. Data Analysis: Total data was subjected to excel statistical analysis for drawing the temperature and time dependent mortality curve as well as calculating the amount of correlation between mortality and duration of temperature ramping.

6. Results and Discussion

Effect of ramping temperature can be prudent for insect pest management. Experiment at three temperature regimes [a] high, [b] moderate [c] low temperature on the survivability of mango mealy bug, *D. mangiferae* was carried out in laboratory condition during 2015. The results are delineated below:

6.1. Survivability in relation to high temperature regime

At 40 ^{0}C (Fig. 3): No visible impact of temperature on the activity of *D. mangiferae* population was noted at 42 ^{0}C if the population kept for first 50 minutes. But the number of living individuals declined at first gradually and then steadily up to 80 minutes. After 90 minutes almost all the population died. Similarly the number of KD population after 60 minutes increased gradually up to 80 minutes but steadily.

At 42 ^{0}C (Fig. 4)**:** At 42 ^{0}C, the number of live individuals declined gradually up to 70 minutes. A drastic fall of the live individuals was noted at 80 minutes. At 90 minutes very few individuals were alive. The number of dead individuals was very low up to 70 minutes. A sudden increase of the dead individuals was noted at 80 minutes. Upto 70 minutes of observation, no major change of KD population was noted. After 70 the number of KD population marginally increased.

At 44 ^{0}C (Fig. 5): A gradual fall of live individuals was noted up to 60 minutes. However most of the population completely died at 90 minutes. The number of KD population increased rapidly up to 50 minutes and then declined rapidly as the number of dead individuals increase.

At 46 ^{0}C (Fig. 6)**:** A rapid and sharp decline of the live individuals was noted up to 30 minutes of observation. However the population completely shattered at 50 minutes. Very few individuals died up to 40 minutes. A sudden increase of dead individuals was noted at 60 minutes. Following that very little alternation of the number of dead individuals was noted. At 90 minutes the total population shattered. The highest number of KD population was noted at 20 minutes. The number of KD individuals then declined at first steadily and then sharply after 40 minutes.

At 48 ^{0}C (Fig.7)**:** All most all the population died at 20 minutes. At 10 minutes, immediately after starting the experiment all of the individuals were knocked down.

At 50 ^{0}C (Fig. 8)**:** Immediately after starting of the experiment almost all the population died.

6.2. Survivability in relation to moderate temperature regime

At 14 ^{0}C (Fig. 9): Very few individuals died at this temperature regime up to 60 minutes. The number of dead individuals increased marginally as the time advances. Following the increase of temperature, most of the individuals were getting knocked down up to 60 minutes of experiment. Initially after 10 minutes of experiment first instances of KD population was noted. KD population gradually increased with time. But starting from 40 to 80 minutes of experiment, no alternation of KD number was noted.

At 18 ^{0}C (Fig. 10): No instances of dead individuals were noted during the entire experiment. Most of the individuals were live during the entire experiment. Number of KD individuals increased though gradually but consistently after 30 minutes.

6.3. Survivability in relation to low temperature regime

At 0 to -4 ^{0}C (Fig. 11): When the population of *D. mangiferae* was kept between 0 to -4 ^{0}C, only very few number of individual were capable to tolerate the temperature stress. Most of individuals died immediately after the exposure. However after 20 minutes of exposure all of the individuals died. Individuals with knockdown situation were not noted.

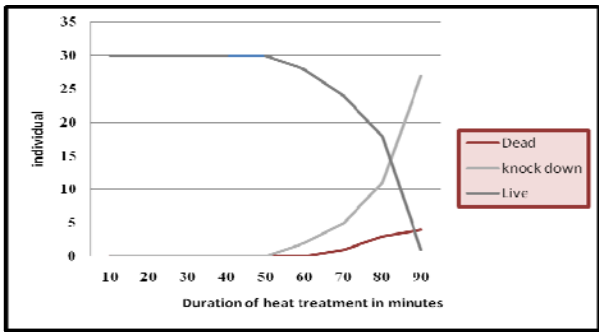

Fig 3: Extent of dead, knockdown and live *D. mangiferae* individuals at 40 ^{0}C

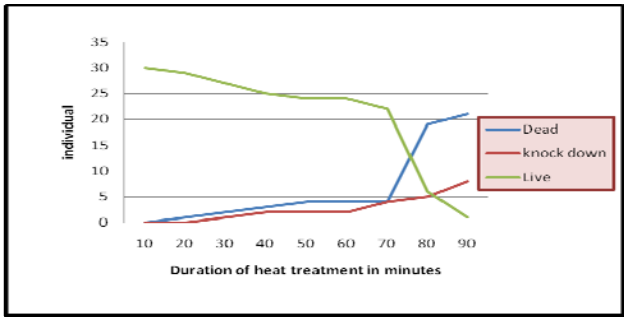

Fig 4: Extent of dead, knockdown and live *D. mangiferae* individuals at 42 ^{0}C

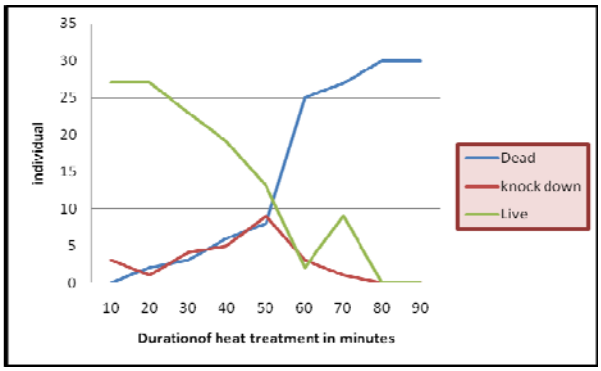

Fig 5: Extent of dead, knockdown and live *D. mangiferae* individuals at 44 ^{0}C

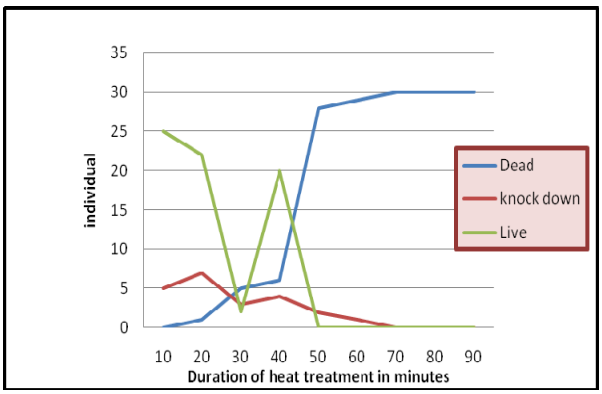

Fig 6: Extent of dead, knockdown and live *D. mangiferae* individuals at 46 ^{0}C

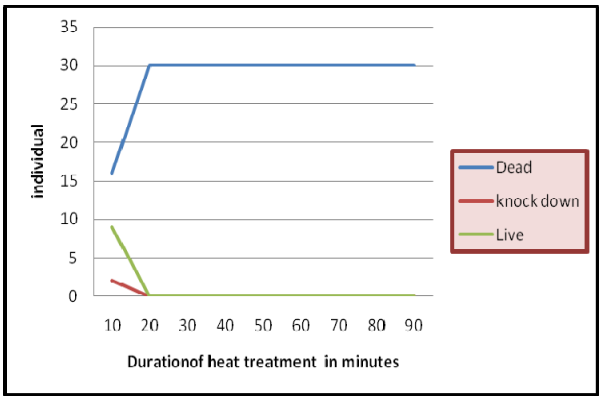

Fig 7: Extent of dead, knockdown and live *D. mangiferae* individuals at 48 ^{0}c

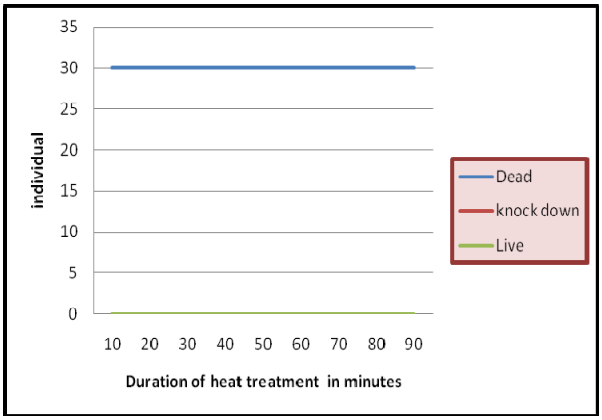

Fig 8: Extent of dead, knockdown and live *D. mangiferae* individuals at 50 ^{0}C

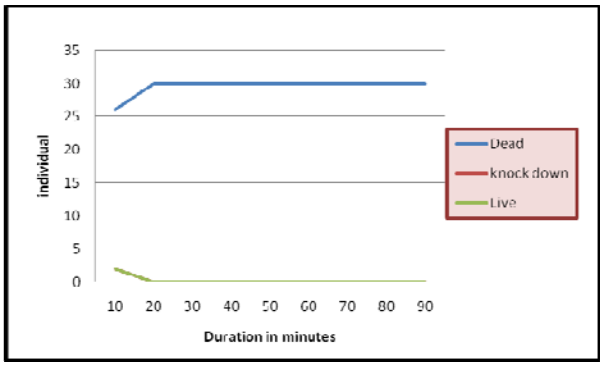

Fig 9: Extent of dead, knockdown and live *D. mangiferae* individuals at -4-0 ^{0}C.

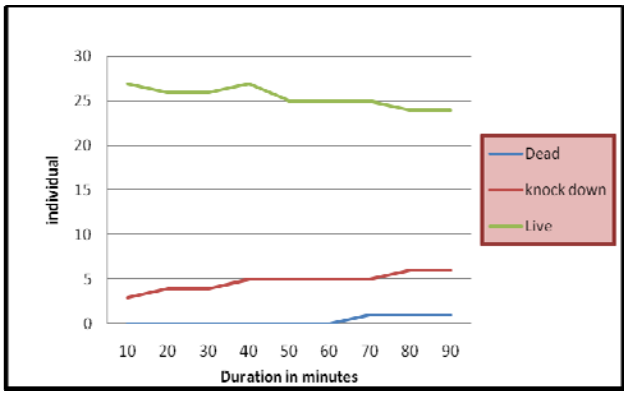

Fig 10: Extent of dead, knockdown and live *D. mangiferae* individuals at 14 ^{0}C.

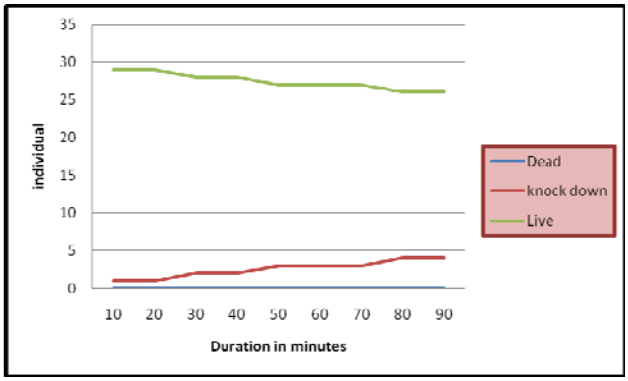

Fig 11: Extent of dead, knockdown and live *D. mangiferae* individuals at 18 ^{0}

Table1: Correlation between death rate (Y) and duration of temperature treatment (x).

Emperature regime	**Temperature (ºC)**	**Regression equation** (Y= insect number, x= given temperature)	**Level of confidence (R)**
Maximum(40 ^{0}-50 ^{0}C)	40	Y=-1.87+0.05413x	0.683**
	42	Y=-7.40+0.26263x	0.569*
	44	Y=-7.02+0.43898x	0.983***
	46	Y=-4.65+0.43602x	0.832***
	48	Y=24.13+0.08280x	0.967***
	50	Y=30.00+0.0000x	0.712**
Moderate(14-18 ^{0}C)	14	Y=-10+0.155565x	0.543*
	16	Y=-10+0.155565x	0.571*
	18	Y=-4.84+0.1461x	0.743**
Low(-4 ^{0}c-0 ^{0}C)	-4	Y=28.32+0.00236x	0.721**
	0	Y=28.32+0.00236x	0.811***

NB: Level of significance; ***maximum (>0.750), **moderate (0.500-0.750) *low (<0.500)

Insects are stenothermal. They show thermo-tolerance either during harsh heat waves or during extended winter. Inconsistent temperature has profound impact on insects' incidence, abundance physiology and behavior [42, 29]. Gilbert *et al.* [14] had shown an almost linear relationship between growth rate and temperature up to about 28 °C followed by a very sharp decline. Present study is in consonance with this finding. Terblanche *et al.* [36, 37] had illustrated that in case of tse tse fly, *Glossina pallipides* alternative change of temperature effects insect behaviour and distresses its activity which are in agreement with the present observation. Abou-Shaara *et al.* [2], on the other hand, have noted that elevated temperature when increased from 30 ^{0}C to 60 ^{0}C at a rate of 0.5 ^{0}C per minute comparatively higher mortality of honeybees was registered. Though the design of the present experiment differs considerably, but the overall result matches with finding of Abou-Shaara *et al.* [2]. The thermal death point is likely to be lower than this the shortest exposure period and lowest temperature tested. In case of our experiments, starting from 40 ^{0}C to 50 ^{0}C, increased mortality was found because at a preset temperature mealy bugs were exposed for a variable period of 10- 90 minutes. Exposure to elevated temperatures has been shown to induce a greater tolerance to subsequent higher temperatures in some insects [10, 38].This induced ability to withstand elevated temperatures is known as the heat shock response [13, 30].

Present observation corroborates to the finding of Woodrow *et al.* [41]. They have noted that termite species *Captotermes formosanus* showed an upward trend of mortality when temperature was elevated from 40 ^{0}C. For *Incistetermis* sp., the number of dead individuals gradually increases from 44 ^{0}c. 100% mortality for *C.formosanus* at 42 ^{0}C and 45 ^{0}C was obtained when the specimens was retained for 90 minutes and 70 minutes respectively. In the present study 100% mortality of mealy bug was obtained when the individuals were exposed for 30 minutes at 44 ^{0}C.

Tang *et al.* [35] presents a thermal kinetic model for 5th instar codling moth larvae based on a thermal-death-time curve for a 20 ºC/min. heating rate which shows 100% mortality after 11 minutes at 48 ºC, 5 minutes at 50 ºC and 1 minute at 53 ºC respectively. The model is supported by the findings of Yin *et al.* [43] that despite thermal conditioning 5th instar codling moth larvae, the most heat resistant stage, suffered 100% mortality after only 3 minutes exposure to 52 ºC. Roti Roti [31] pointed out that elevated temperature deforms body building macromolecules which ultimately lead to the death of the organism. However, Bowler [6] have pointed out that damage to cell membrane at high temperature consequences to the death of organism. Eggs of the Indian meal moth (*Plodia interpunctella*), a stored products pest, were found not to survive at 48 °C for 34 minutes [27]. Similarly, Hosking [15] had shown that diapausing eggs of gypsy moth (Lymantriidae) suffered 100% mortality after 5 minutes exposure at 55 ºC. Gould *et al.* [16] found that hot water immersion at 49 °C for 20 minutes was lethal to the mealybugs *Planococcus citri* and *Pseudococcus odermatti* on limes. They also noted that the treatment also killed all other arthropods found externally on limes, in a study involving 7200 fruits. Kersting *et al.* [24] found that continuous exposure to 35 °C was lethal for the aphid *Aphis gossypii.* Insect death by heat shock results from protein denaturing and associated effects on enzymes [12] an effect which probably accounts for the relative consistency of the thermal mortality threshold.

On the other hand low temperature regime also effects insects mobility [4, 5]. Constant low temperature in comparison to fluctuating thermal regime (FIR) is comparatively more detrimental to insect individuals [9]. Kostal *et al.* [25] had shown that *Pyrrhocoris apterus* and *Alphitobius diapterinus* developed chill injury more rapidly when they were exposed to constant low temperature. In the present study *D. mangiferae* did not show any sign of such injury at 14 ^{0}c or 18 ^{0} C. It was only noted when *D. mangiferae* are exposed at 14 ^{0}C for 70-80 minutes, 3.3% of the total population under experimentation showed the sigh of wilting but that result was statistically very insignificant. But at subzero temperature -4 ^{0} to 0 ^{0}C. 86.7% and 100% dead was noted when the individuals were kept for 10 minutes and 20 minutes respectively.

After hatching 1st instar nymph of mango mealy bug about to appear during December-January [7]. The agro-climatic situation is conducive for the survival and development of the instar as the gross maximum and minimum temperature fluctuates between 25 ^{0} c to 15 ^{0}c further very low mortality at 14 ^{0}C and 18 ^{0}C are in agreement with their natural conditioning. But zero to subzero temperature is very lethal to them. Tentatively, optimum temperature ranges between 15 ^{0}C to 40 ^{0}C and based on this temperature assay pest incidence, decline or reaching their peak number, range of distribution can be predicted and accordingly control measures can be planned.

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