RESEARCH PAPER | Zoology | Volume : 4 | Issue : 1 | Jan 2014 | ISSN - 2249-555X

Sterilization of honey bee combs infected with *Melissococcus plutonius* through chemicals and UV rays

KEYWORDS: Melissococcus plutonius, sterilization, chemicals, UV rays, bee combs

Deepak Singh	Dr. Sapna Katna	Dr. B. S. Rana
Vill. Upper Lahasa, P O Rampur Bushaher, Distt. Shimla HP 172001	Astt. Professor, Department of Entomology, Dr Y S Parmar University of Horticulture & Forestry, Nauni, Solan, H P	Sr. Entomologist, Department of Entomology, Dr Y S Parmar University of Horticulture & Forestry, Nauni, Solan, H P

ABSTRACT *The causal bacterium, Melissococcus plutonius of European foulbrood disease was isolated from infected larvae of A. mellifera which inhabits the combs. Such combs are source for further spread of infection. During present studies, artificially inoculated (with M. plutonius) frames were sterilized by treating with chemicals viz., formalin, detergent, sodium hypochlorite, hydrogen peroxide + iodine monochloride and UV rays for different time exposures. Maximum mortality of M. plutonius (99.00%) was observed in 4% formalin when exposed for 8.0 min. While 1% formalin + 2% detergent treatment for 8.0 min gave 97.66% mortality. Irradiation with UV rays for 20 min exposure caused 91.02% mortality of M. plutonius. The colonies of A. mellifera gave normal response on the treated combs. Thus these studies indicate that the sterilization of M. plutonius infected bee combs with 1% formalin + 2% detergent is very effective, beekeeper friendly with low cost and minimum residual effect.*

INTRODUCTION

Honey bees are social insects of great scientific importance. These provide valuable products and also play a vital role in pollination of crops and maintaining their biological diversity (Jahannesmeir and Mostert, 2001) and continuity (Free, 1960). Health and vigour of honey bee colonies are threatened by numerous enemies and diseases caused by viruses, bacteria, fungi, protozoa and mites all of which render the colonies too weak (Bailey, 1981). European foul brood disease is one of the important bacterial diseases which is prevalent throughout the country in both the hive bees (Rao *et al.*, 2011). It mainly affects larvae less than 48h of age. In India, it was recorded for first time in *A. cerana* from Kharad area of Maharashtra during 1970 (Diwan *et al.*, 1971) which killed 25-30 % of colonies. In *A. mellifera,* it was noticed in Himachal Pradesh (Anonymous, 1998). The causative bacterium *Melissococcus plutonius* is non-spore forming, gram positive and lanceolate in shape occurs mostly in chains (Bailey and Collins, 1982). The microorganisms like viruses, bacteria, fungi and protozoa after infecting brood and adult bees remain in combs and equipment as source of the inoculum. Such combs are source for further spread of infection. Even after controlling the disease by treating the honey bee colonies with antibiotics or other chemicals, the infections reappear after sometime. To overcome this problem, combs and equipments needs to be sterilized to prevent further infection.

Thus keeping in view the destructive nature of the micro-organisms inhabiting bee combs and extent of losses to apiculture industry, the present investigations were carried out to study the effect of some chemicals & UV rays on the viability of *M. plutonius* from *Apis mellifera* L. combs and also to study the acceptance of treated combs by honey bees.

MATERIALS AND METHODS

Inoculation

Dry and empty freshly raised to one year old combs/frames from *A. mellifera* colonies were selected for inoculation of *M. plutonius*. Two marked areas of 50 worker cells on each side of frames were filled with bacterial suspension counting 30000±200cfu/20µl and air dried. One control was kept in each treatment. Sterilization of these combs was carried out by treating/dipping the combs with different concentrations of formalin (1.0, 2.0, 4.0 %), formalin plus detergent (1.0% +1 % , 1.0% + 2.0%), detergent alone (1.0, 2.0 %), sodium hypochlorite (20, 50, 100, 200, 450 ml/ hive body) and hydrogen peroxide + iodine monochloride in the ratio 10:1 (20, 50, 100, 200, 450 ml/ hive body) at different time intervals. Each treatment was replicated 4 times.

Sterilization of combs was also done by irradiating the combs with UV light (20 watts) for 2, 5, 10, 15, 20 min separately.

Extraction

After treatments, out of 50 comb cells in each treatment and replication, sample from 5 comb cells from each marked area were collected randomly. Finally these 5 extracted samples were pooled to count down the numbers in 0.20µl/sample under haemocytometer. Recovery of bacterial cells/ 20µl was worked out in each treatment. Viability of bacterium was observed by growing in specific basal medium (Rao *et al.*, 2011). After 72 h of incubation, number of bacterial colonies showing characterstics of *M .plutonius* were counted to work out per cent mortality with following formula:

$$\text{Percentage mortality} = \frac{\text{Reduction in no. of viable cells}}{\text{Total no. of viable cells}} \times 100$$

Acceptability

Acceptance of treated combs (those treated with highest dose of different treatments) was checked by giving them in *A. mellifera* colonies. Before use, the treated combs should be washed thoroughly with water. Data on number of bees covering the frames, egg laying in treated areas, hatchability (%) and sealing of brood (%) were recorded.

Data collected from experiments conducted in field and laboratory was statistically analyzed with the help of Analysis of Variance in CRD.

RESULTS AND DISCUSSION

The percent mortality of *M. plutonius* increased from 86.75 to 89.77 with the increase in concentration of the mixture i.e 1.0% formalin + 1% detergent to 1% formalin + 2% detergent (Table 1). When the duration of dipping of the inoculated combs increased from 2.0 to 8.0 min, significantly it increased from 81.42 to 97.66. In the present studies, the

Volume : 4 | Issue : 1 | Jan 2014 | ISSN - 2249-555X

highest mortality of bacterium (97.66%) was recorded by dipping the combs in 1% formalin + 2% detergent for 8.0 min. It means that up to 100.00% mortality or sterilization can be achieved by dipping the combs in 1.0% formalin + 1% detergent for longer duration upto 1.0 h. It will reduce the cost of the sterilization as well as the residual effect.

Table 1. Effect of different concentrations of formalin + detergent and detergent alone on per cent mortality of *M. pluton*. Recovery (100±10cfu/0.2µl)

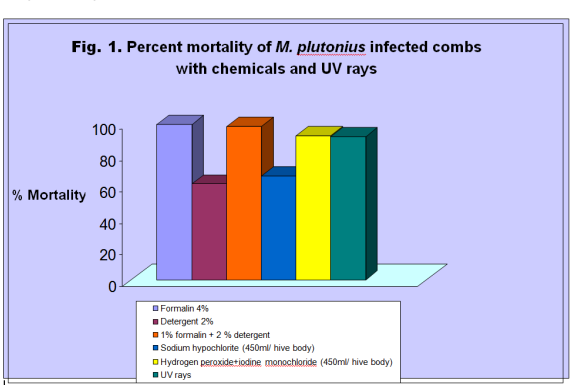
Treatments	Per cent mortality of *M.pluton* after different time exposure :				
	2 min	3 min	5 min	8 min	Mean
1.0% formalin + 2.0%detergent	81.42 (64.47)	87.25 (69.09)	92.75 (74.39)	97.66 (81.27)	89.77 (72.30)a
1.0% formalin + 1.0% detergent	76.83 (61.23)	84.50 (66.82)	90.08 (71.65)	95.58 (77.89)	86.75 (69.40)b
1.0% detergent	35.25 (36.42)	43.17 (41.07)	48.00 (43.85)	59.00 (50.52)	46.35 (42.18)d
2.0% detergent	48.50 (44.14)	52.25 (46.29)	59.91 (50.72)	61.08 (51.41)	55.44 (48.14)c
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)e
Mean	48.40 (41.25)d	53.43 (44.65)c	58.15 (48.12)b	62.67 (52.15)a	

Values in parentheses are arc sine transformed values

$CD_{0.05}$

Treatments (T) = 0.64, Exposure time (E) = 0.57, T x E = 1.28

With the increase in concentration of formalin from 1.0 to 4.0% and duration of treatment from 2.0 to 8.0 min, mortality of *M. plutonius* in the inoculated combs significantly increased from 54.87 to 92.89 % (Fig. 1). Irrespective of the concentrations of formalin, when the duration of dipping of the combs was increased from 2.0 to 8.0 min, the per cent mortality of *M. plutonius* significantly increased from 50.14 to 63.48. No studies have been conducted with formalin on the sterilization of combs and bee equipments. However, studies on formaldehyde drenching for the control of *Fusarium* wilt had been carried out by O'Neill *et al.* (2005). Formaldehyde was applied @ 0.5 l/ m^2. The treatment reduced the viability of *F. oxysporum* from 97% to 41% at 0 and 15 cm, depth respectively.



By fumigating the combs with sodium hypochlorite @450ml/ hive body for 5 h maximum mortality of 66.22% was recorded (Fig1). Whereas the minimum mortality (39.00%) was observed with a dose of 20ml fumigant/hive body in 0.5 h of fumigation. Earlier, Wilson (1924) had also conducted the unsuccessful preliminary studies on the sterilization of combs infected with American foul brood disease by using sodium hypochlorite.

The mortality of the bacterium was significantly increased from 53.84 to 83.18 per cent with the increase in doses of hydrogen peroxide +iodine monochloride from 20 to 450 ml/ hive body. Similarly, with the increase in duration of fumigation from 0.5 to 5.0 h, the mortality significantly increased from 48.87 to 62.85 % (Fig. 1). However economically, this treatment is very costly because iodine monochloride is very expensive, can't be easily adopted by beekeepers. Earlier, Smirnov (1974) had also effectively sterilized the combs contaminated with American foul brood (*Paenibacillus larvae*) and European foul brood diseases through the fumigation with hydrogen peroxide + iodine monochloride in fumigation chamber.

Exposure of combs under UV rays for 20 min caused maximum mortality (91.20%) of *M. plutonius* (Fig.1). Per cent mortality of *M. plutonius* or per cent sterilization of treated frames increased with the increase of exposure duration under UV rays. So far, no study has been conducted on the use of UV rays for the sterilization of bee equipments and the combs. However, it is being frequently used in sterilization of medical instruments (Morris, 1972). The sterilization with UV rays is residue free, cheaper and effective, provided the surfaces are dry and directly irradiated (Gendemann and Mangum, 2001).

The data collected on the acceptability of treated combs by honey bees indicate that *A. mellifera* showed similar response in treated as well on the control/untreated combs (Table 2). Further these findings revealed 1.0 to 5.0% mortality in both the treated and untreated comb cells which was a normal phenomenon.

Table 2. Response of *A. mellifera* on the treated combs with respect to some biological characters

Treatment	Exposure/ Treated duration	Response of *A. mellifera* on the combs with regard to			
		No. of bees/ frame	Egg laying (%) / in treated area	Hatchability (%)	Sealing of larvae (%)
Formalin solution (4%)	8 min	2340.00	93.50	89.00	89.00
Sodium hypochlorite (450ml/hive body)	5h	2400.00	95.50	93.50	92.50
Hydrogen peroxide+ iodine monochloride (450ml/hive) body)	5h	2100.00	95.00	89.50	88.50
UV rays	20 min	2700.00	96.50	95.50	95.50
Control	5h	2680.00	96.00	94.50	93.50

These studies corroborate with earlier findings of different research workers who had also recorded 4.93 to 16.94% brood mortality rate which is normal in *A. mellifera* colonies in North India (Mishra and Kumar,1995; Anonymous 1996; Chaudhary *et al.*,1998). According to Ruttner (1988) more than 25% mortality of brood is hazardous for the productivity of colonies.

Volume : 4 | Issue : 1 | Jan 2014 | ISSN - 2249-555X

REFERENCE

Anonymous, 1996. Annual Report. All India Co-ordinated Project on Honey Bee Research and Training. Department of Entomology, PAU, Ludhiana 22p. | Anonymous, 1998. Studies on control of natural enemies and diseases of honey bees. Annual Progress Report, deptt. of Entomology & Apiculture, Dr. Y. S. Parmar university of Horticulture and Forestry, Nauni, Solan. pp. 45-46. | Bailey, L. 1981. Honey Bee Pathology. Academic Press Inc., London. | Bailey, L. and M. D. Collins. 1982. Taxonomic studies on Streptococcus plutonius. J. App. Bacteriol., 53:209-213. | Chaudhary, S., J. K. Gupta and B. S. Rana. 1998 Variation in Worker brood survival of A. mellifera L. Pest Manag. Econ. Zool., 6(1): 65-67. | Diwan, V. V., K. K. Kshirsagar, R. A. V. Raman, D. Raghunath, , C. S. Bhambure and S. H. Godbole. 1972 Occurrence of new bacterial disease of Indian honey bee Apis indica F. Curr. Sci., 40: 196-197. | Free, J. B. 1960. The behavior of honey bees visiting flowers of fruit trees. J. Anim. Ecol., 29: 385-395. | Gendemann, B. G. and S. S. Mangum. 2001. Ultra-violet radiation and lights in infection, prevention in surgical settings. W B Saunders Company : Philadelphia, pp 32-35. | Johannesmeir, M.F. and A.J.N. Mostert. 2001. Crop pollination, in: Johannesmeir, M. F. (Ed), Beekeeping in South Africa. Pretoria Agricultural Research Council, pp. 235-250 | Mishra, R. C. and Y. Kumar. 1995. Introduction and spread of A. mellifera L. and possibility of inbreeding harm. Indian Bee J., 57: 130-134. | Morris, E. J. 1972. The practical use of ultra-violet radiation for disinfection purposes. Medl. Lab. Technol., 29 (1): 41-47. | O'Neill, T. M., K. R. Geoen and T. Ratcliffe. 2005. Evaluaton of soil steaming and a formaldehyde drench for control of Fusarium wilt in column stock. Acta Horticulture 698: 129-133. | Rao, K. M., B. S. Rana, S. K. Chakravarty, Sapna Katna and Anju Sharma. 2011. Characterization of Melissococcus pluton from European honey bee (Apis mellifera L.) of North- West Himalayas. Int. J. Sci. Nat., 2 (3): 632-638. | Ruttner, F. 1988. Breeding technique and selection for breeding of honey bees. The British Isle Bee Breeders Association, Derby, UK, 152p. | Smirnov, A. M. 1974 Disinfection of combs and foundation with solution of hydrozen peroxide and iodine monochloride for American and European foulbrood. Veterinariya, 8: 109-111. | Wilson, H. F. 1924 Sodium hypochlorite treatment; the germicidal action of disinfectant on the spores of American foulbrood. Gleanings Bee Culture, 52: 648-649.