

PHYSICAL AND BIO-CONTROL METHODOLOGIES FOR INTEGRATED MANAGEMENT OF BUFFALO FLY-*Haematobia exigua* (DIPTERA: MUSCIDAE)

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## ABSTRACT

*Haematobia exigua*, the buffalo fly, is a common ectoparasite of buffaloes and cattle in India causing economic losses and underlines the necessity of effective control strategies against them. The present study was planned to evaluate larvicidal activity of few methods for physical and biological control for integrated management of *Haematobia exigua* flies. Out of four bacterial bio-control agents evaluated by *in-vitro* assay and Probit analysis, two bacteria i. e. *Bacillus thuringiensis* var *israelensis* and *Bacillus thuringiensis* var *kurstaki* showed excellent larvicidal activity against *H. exigua* with LC<sub>50</sub> value of 134 and 135 mg per liter of water respectively. Whereas, other two bacteria i. e. *Bacillus weihenstephanensis* WSBC and KBAB4 failed to show desired larvicidal activity. It can be concluded that *B ti* and *B tk* have future as effective bacterial agents (BCAs) in the control and integrated management of buffalo and horn fly. An experiment was conducted in the laboratory and on field for judging the efficacy of a physical method of tightly covering dung pats with polythene sheet for control of larvae of *H. exigua*. The laboratory result showed only 14.73% larvae were survived and developed per 100 gm of the faeces

in the pot covered with polythene sheet as against significantly higher number 78.33% in the control group. During field trial, average larval count from dung pits before the experiment was 86.33 per 250 gm of faeces and it has been significantly reduced to 11.80 after covered with polythene sheets for two weeks. It indicated that physical method of covering the dung pits works immensely and thus can be inducted in IPM program as one of the effective physical control alternatives.

**Keywords:** *Bubalus bubalis*, buffaloes, *Haematobia exigua*, bacterial bio-control agents, integrated pest management

## INTRODUCTION

*Haematobia exigua*, the buffalo fly, is a common and economically important ectoparasite of buffaloes and cattle in India, Indonesia, Malaysia, Papua New Guinea, China and northern Australia (Foil and Hogsette, 1994). In the United States, horn fly, a close relative of buffalo fly causes annual losses as much as US\$ 1 billion and an additional US\$ 60 million is spent annually on

insecticides to control infestation (Byford *et al.*, 1992). In high-grade infestations, there is 10 to 20% reduction in milk production by dairy cows (Metcalf and Metcalf, 1993; Williams *et al.*, 1985). *Haematobia exigua* act as biological vector for the filarial nematode *Stephanofilaria* spp. including *S. stilesi* and *S. assamensis* (Foil and Hogsette, 1994; Shaw and Sutherland, 2006; Saparov *et al.*, 2014).

Most of the control strategies against *Haematobia* flies involve the use of chemical insecticides. Emerging problems of insecticide resistance, insecticide residues in animal products and its toxic effects, are serious issues concerning to livestock industry. An alternative to conventional insecticide is integrated pest management (IPM) practices which involves the utilization of safe and economically effective biological control agents such as bacterial biocontrol agents and compounds derived from many plants. Past investigations also explored pesticides derived from herbal origin such as Neem, *Eucalyptus*, *Pongamia* etc. against *Haematobia* flies as alternative control (Gudewar *et al.*, 2020). Temeyer (1984) reported the larvicidal property of a strain of *Bacillus thuringiensis* subsp. *israelensis* and of *Bacillus thuringiensis* subsp. *kurstaki* against horn flies, *Haematobia irritans* (Diptera: Muscidae); while Pinnock (1994) in a review article stated that horn fly larvae are susceptible not only to thuringiensin but also to other toxins produced by *Bt* var. *israelensis*. Physical control, though offering great potential as a component of integrated control systems and can completely replace current chemically-based protection methods, is not yet widespread for use against field pests (Banks, 1976).

The role of these flies as pest causing economic losses and having potent vector capacity underlines the necessity of research on effective control strategies against them. Considering

the early observations highlighting the success of physical, herbal and bacterial BCAs against muscoid flies, the present study was planned to evaluate larvicidal activity of few methods for physical and biological control of *Haematobia exigua* flies.

## MATERIALS AND METHODS

### Collection of *Haematobia exigua* larvae

For the present experiment, field collection of larvae was carried out from the faecal samples collected from dung pits belonging to different buffalo shed of private dairy owners nearby Parbhani city. In the laboratory, faecal samples were diluted with three times water quantity, filtered with strainer and from the processed samples larvae were individually picked up with moist camelin hair brush of size 1. Few larvae were processed for identification under zoom stereoscopic microscope by applying keys (Baker, 1987; Fitzpatrick and Kaufman, 2011) before being included in the experiment

### Bacterial bio-control agents

The bacterial lyophilized powder/culture containing spores was used in the present experiment. The bacterial culture, in the lyophilized form were available in the Department of Veterinary Parasitology, College of Veterinary and Animal Sciences, Parbhani. These bacteria were isolated and stored during the DBT financed research project on IPM that was completed in the department during the year 2012 (Table 1). However, *Bacillus thuringiensis* var *kurstaki* was purchased from with the trade name Dipel-8L containing spores + toxin manufactured by Valent Biosciences corporation, USA. All these agents

were first grown in nutrient broth to confirm their viability. A drop of culture was taken on a glass slide, heat fixed and stained by Gram staining to check the presence of bacilli. After confirmation, all these BCAs were used in the research to compare their efficacy against larval stages *Haematobia exigua*.

### **Bio-assay procedure for larvae of *Haematobia exigua* flies**

For *in-vitro* trial with bacterial agents and toxin, fresh faecal sample were collected from the buffalo farm of College of Veterinary and Animal Sciences, Parbhani. Isolated 10 larvae were inoculated on faecal pods in Petri dishes. Such five Petri dishes were inducted for each concentration of every bacterial agent and toxin. *Bacillus thuringiensis* var *israelensis*, *Bacillus weihenstephanensis* WSBC and *Bacillus weihenstephanensis* KBAB4, stock solution was prepared by mixing 0.1 gm lyophilized powder of each bacteria in 5 ml normal saline solution (NSS) as stock solution. From this stock solution, 1 ml was diluted in 9 ml of NSS as first dilution. Similar way 1 ml from first dilution was diluted in next 9 ml NSS. Likewise five serial dilutions were prepared in descending grades and used in the present experiment. *Bacillus thuringiensis* var *kurstaki* was commercially available as Dipel-8L liquid. Each ml of it contains mixture of bacterial spores and toxin 17600 IU per mg and liquid is prepared as 3.5% W/W solution. From Dipel-8L liquid 1 ml was diluted in 9 ml distilled water and used as first concentration. And likewise five serial dilutions were prepared in descending grade in distilled water and used in the present experiment. All five serially diluted concentrations were sprayed on the faecal culture seeded with 30 larvae per 50 gm of faeces in Petri dish. Control group was maintained

for each bacterial culture as 30 larvae in 50 gm of faeces and sprayed with normal saline solution. All the petri dishes consist of treatment and control group were covered with plastic lids containing fine mesh net to avoid breeding of any other insect in the faecal media. All the trials were repeated thrice and average mortality count was recorded as assessment criteria for judging the efficacy of bacterial agent. The observations were taken till the period, when all the larvae in control group molted to pupal stage. The individual larvae were considered as dead when it showed no movement even after pricking with pinhead. The criteria for assessment of efficacy of bacterial control agents was mortality of larvae. The mortality data was tabulated and efficacy was worked out in terms of percent mortality.  $LC_{50}$  values were worked out by using the method described by Finney, (1952) using Microsoft excel software (Sharma *et al.*, 2018) and then comparison was done with  $LD_{50}$  values of acute toxicity in rats referred from literature.

### **Physical method for control of larvae of *Haematobia exigua***

#### **Laboratory trial**

An experiment was conducted in the laboratory as *in-vitro* trial for judging the efficacy of a physical method for control of larvae of *Haematobia exigua*. From the dung pats (manure heap-cum-breeding site) containing larvae of *Haematobia exigua* flies, 100 gm of faecal sample was collected in a ten Petri dishes (each contains 100 gm) and larval count in each Petri dish was noted at the beginning of experiment. Five such Petri dishes were tightly covered with polythene sheet (treatment group) and five Petri dishes were covered with net (control group) and maintained in the laboratory for two weeks. After 14 days, larval count in 100 gm faecal sample from all ten

faecal samples in the Petridish was recorded. The difference in the larval survival percent in the treatment and control group was taken into account for judging the efficacy of physical method under evaluation. Such trials were repeated thrice and the mean survival rate was worked out by applying descriptive statistics and results were tabulated.

### Field trial

A field trial was conducted for judging the efficacy of a physical method to control larvae of *Haematobia exigua* flies at the animal farm of College of Veterinary and Animal Sciences Parbhani. The five dung pats (manure heap-cum-breeding site) containing larvae of *Haematobia exigua* flies were utilized for field trial. From each dung pat, 250 gm of faecal sample were collected and larvae present were counted manually (pretreatment- Day 0). For the trial, a transparent large plastic sheet was cut into 2x2 meter pieces and five dung pats marked for trial were covered with the piece of plastic sheet tightly. The experiment was carried out for two weeks. After that, larval count in 250 gm faecal sample from all five dung pats (posttreatment- Day 14) was recorded. The pre and post treatment difference in the larval count of the same dung pat was taken into account for judging the efficacy of physical method under evaluation. Such trials were repeated thrice, and the mean mortality was worked out by applying descriptive statistics using computer application, WASP version 2.0 ([www.ccari.res.in](http://www.ccari.res.in)) and results were tabulated.

## RESULTS AND DISCUSSIONS

### Evaluation of bacterial bio-control agents against larvae of *Haematobia exigua* flies

The results of the present study are summarized in Table 2 and Probit analysis graphs (Figure 1 and 2). Four bacteria have been evaluated against larval stages of *Haematobia exigua* flies. The LC<sub>50</sub> value for these four bacterial bio-control agents viz. *Bacillus thuringiensis* var *israelensis*, *Bacillus thuringiensis* var *kurstaki*, *Bacillus weihenstephanensis* WSBC and *Bacillus weihenstephanensis* KBAB4 was 134, 135, 275696 and 1159561 mg per liter of water, respectively. From the experiment and LC<sub>50</sub> value calculated it has been observed that two bacteria viz. *Bacillus thuringiensis* var *israelensis* and *Bacillus thuringiensis* var *kurstaki*, were found to have good larvicidal activity; while two bacteria viz. *Bacillus weihenstephanensis* WSBC and *Bacillus weihenstephanensis* KBAB4, not yielded expected percent of mortality of larvae and were required in very high concentration. Owing to these fact, present study opines that, later two bacteria cannot become part of integrated pest management module.

In the present study few serovars of *Bacillus thuringiensis* has been tested and have given the encouraging results against *Haematobia exigua* buffalo fly larvae. In the literature quantum of research has been undertaken on bacterial control of *Haematobia exigua* buffalo flies, which is discussed in this topic. *Bacillus thuringiensis* (Berliner) (*B. t*) is a naturally occurring bacterium that produces insecticidal proteins that are active against various insects. A variety of subspecies exist, the two most commonly used are *B. t. kurstaki* for control of Lepidoptera and *B. t. israelensis* for control of mosquitoes and black flies and some activity against horn fly larvae (Lysyk *et al.*, 2010). Temeyer (1984) reported that a strain of *Bacillus thuringiensis* subsp. *israelensis* was found larvicidal against the larvae of horn

flies, *Haematobia irritans*. Concerned to it, author noted a significant finding as toxicity of *Bti* against horn fly larvae is independent of vegetative cells or endospores and is due to production of toxic substances rather than to bacteremia. Haufler and Kunz (1985) reported that mortality of treated horn fly larvae was dependent on concentration of lyophilized toxin. The greatest mortality occurred in the pupal-adult transformation. Due to toxin pupae were affected and they became smaller size and lighter in colour compared to pupae from Control group. Gough *et al.* (2002) conducted studies on efficacy of *Bacillus thuringiensis* against buffalo fly larvae. The assay author observed that around 96 *Bt* isolates had high toxic activity against buffalo fly larvae and affected larvae failed to successfully pupate. Similar observations noted in the present study in which *Bt israelensis* Treated group up to 86% and in *Bt kurstaki* group up to 83% larvae failed to successfully pupate. Lysyk *et al.* (2010) reported the five highly active isolates *viz* *B.t. tolworthi* 4L3, *B.t. darmstadiensis* 4M1, *B.t. thompsoni* 4O1, *B.t. thuringiensis* HD2, and *B.t. kurstaki* HD945 highly toxic to horn fly immatures. From all observations including present study the conclusions can be drawn as; *B ti* and *B tk* have future as effective bacterial agents (BCAs) in the control and integrated management of not only buffalo and horn fly but also for many dipteran pests of veterinary importance. *B.t. kurstaki* previously considered as specific to Lepidoptera pests, has found as promising against dipteran pests.

#### **Evaluation of physical control method against larvae of *Haematobia exigua* flies**

The laboratory trial results summarized in the Table 3 indicated that significantly higher number (78.33%) of the larvae per 100 gm of the faecal matter was noted as surviving and

developing when faecal matter was not covered with polythene sheet and larvae were having open access to the air (control group). While larvae in the pot covered with polythene sheet has been died due to hypoxia and only 14.73% larvae were survived and developed as against 78.33% in the Control group.

Similar type of the experiment was carried out in the natural environment as field trial. During trial dung pits in which recently discharged faecal matter was dumped has been covered with polythene sheets. From these dung pits before the experiment average larval count was 86.33 and it has been significantly reduced to 11.80 per 250 gm faecal matter. It indicated that physical method of covering the dung pits works immensely and helps in reducing the larval population drastically (Table 4).

Freshly laid faecal matter (on which buffalo fly lays the eggs) is collected and dumped in the dung pit as a routine managerial practice on each dairy farm. In the dung pit larvae hatches from eggs, feed on bacteria in the dung and receives oxygen through respiration by facing the spiracles towards the environment in the open sky. In such dung pit airtight environment has been created artificially by using polythene sheet as physical method. Due to such physical barrier the emerging larvae get deprived from respiration and their development either get delayed or death occurs on the account of hypoxia. Physical control method tested *in-vitro* and on field in the present study has been probably studied for first time. The results after undertaking field trials were encouraging and thus can be inducted in IPM program as one of the effective physical control alternatives. For comparison of our study no such other literature is available. Therefore, efforts are made to have discussion on other physical methods developed

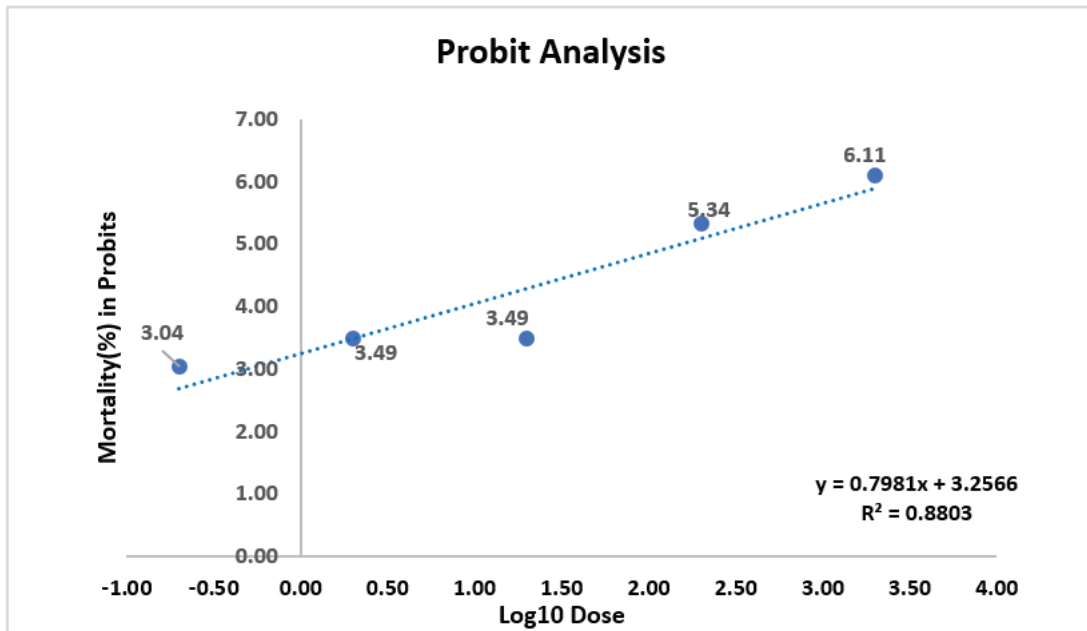


Figure 1. Probit analysis,  $LC_{50}$  and Fiducial limits for larvicidal effect of *Bacillus thuringiensis* var *israelensis* against larvae of *Haematobia exigua*.

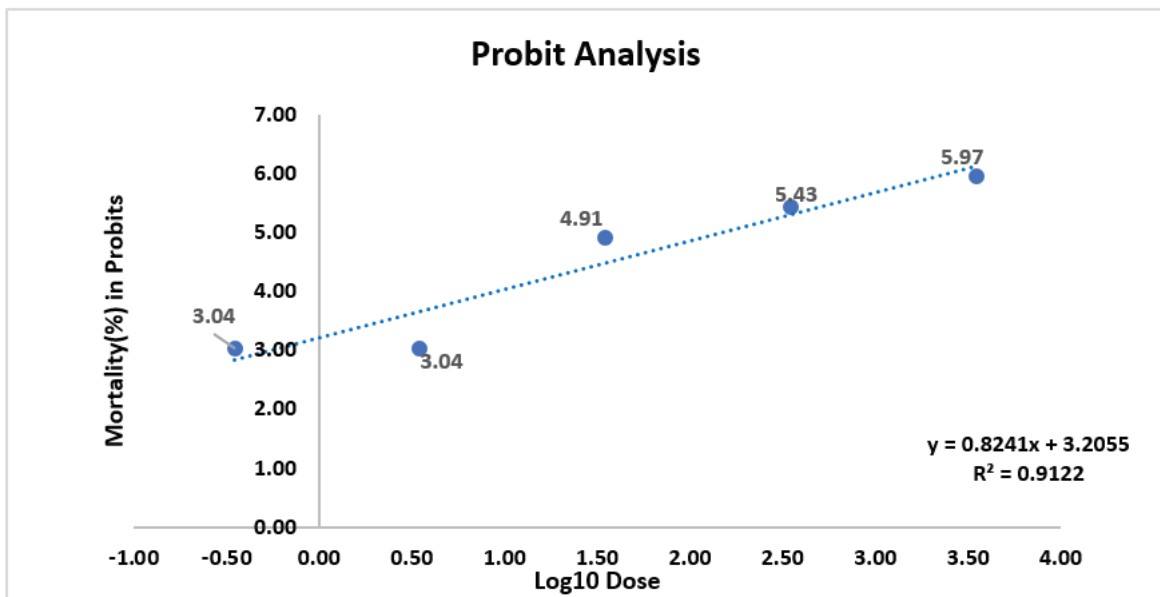


Figure 2. Probit analysis,  $LC_{50}$  and Fiducial limits for larvicidal effect of *Bacillus thuringiensis* var *kurstaki* against larvae of *Haematobia exigua*.

Table 1. Different bacterial cultures used in the present experiment.

Sr. No.	Scientific name of the bacteria	Concentration
1	<i>Bacillus thuringiensis var israelensis</i>	Lyophilized powder $2.0 \times 10^{10}$ spores per gram.
2	<i>Bacillus weihenstephanensis</i> WSBC	Lyophilized powder $1.3 \times 10^{10}$ spores per gram.
3	<i>Bacillus weihenstephanensis</i> KBAB4	Lyophilized powder $1.3 \times 10^{10}$ spores per gram.
4	<i>Bacillus thuringiensis var kurstaki</i>	Dipel-8L liquid each ml contains 616000 IU.

Table 2. Lethal concentrations of the different bacterial biocontrol agents against larval stages of *Haematobia exigua* at 95% confidence limits.

Bacterial BCA	LC <sub>50</sub> (mg/litre)	95% Fiducial CI		Slope $\pm$ SE	$\chi^2$	n
		Lower	Upper			
<i>Bacillus thuringiensis var israelensis</i>	134.383	37.741	478.488	0.798	0.744	30
<i>Bacillus thuringiensis var kurstaki</i>	135.659	39.929	460.903	0.824	0.768	30
<i>Bacillus weihenstephanensis</i> WSBC	275696	9885.226	7689125	0.358	0.870	30
<i>Bacillus weihenstephanensis</i> KBAB4	1159561	25912	51889554	0.323	0.911	30

Table 3. Survival rate (%) of larvae in the faecal matter after application of Physical method- Laboratory studies (n=15).

Physical method	Mean	Range	't' Value	't' Table (0.05)	't' Table (0.01)	Significance at 5%
Faecal pods covered with polyethene sheet	14.73 $\pm$ 1.20	8-24	48.87	2.14	2.97	Significantly different at both 5% and 1% level of significance
Faecal pods not covered with polyethene sheet (control)	78.33 $\pm$ 0.54	76-81				

Table 4. Survival rate (%) of larvae in the faecal matter after application of Physical method-Field studies (n=15).

Physical method	Mean	Range	't' Value	't' Table (0.05)	't' Table (0.01)	Significance at 5%
Count of larvae in 250g of faeces from dung pits before covering of dung pit with polythene sheet	86.33 $\pm$ 4.70	65-127	17.67	2.14	2.97	Significantly different at both 5% and 1% level of significance
Count of larvae in 250g of faeces from same dung pits after three weeks	11.80 $\pm$ 1.16	5-22				

against larvae of horn flies.

Du Toit (1938) described the method of destruction of the larval stages as-a) disposal of manure over large areas so that faeces will dry off before the larvae completes their development, b) disturbance of the faeces so that air will enter in faecal pad and will rapidly dry it, making unsuitable for further development of larvae. Foil and Hogsette (1994) suggested destruction of manure pat integrity, the other type of physical control for destruction of larvae of horn flies. Banks (1976) while dealing with pests of crops, described in detail about fundamentals of physical control of any insect pest. According to him physical control of insect pests has become prominent recently because of a) development of resistance to pesticides, b) need to avoid insecticide residues in feed and c) economic reasons. Long term physical control provides good alternative when integrated with cultural methods, genetic control, biological or behavioral methods and as last resort chemical pesticides. He described that physical control means and includes alteration of the environment by any physical means to make it hostile or inaccessible to the pest insects. During the present study, use of polythene sheet to cover the dung pit for making alteration in environment and depriving the larvae from respiration has resulted in encouraging results and emerged as newer type of physical control method.

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