

Title: Efficacy Evaluation of Poultry spray® on control of
Alphitobius diaperinus (Darkling beetles) in Turkeys

Field Efficacy Study

Dr. Manjunatha Mahabalarao, DVM MAHM
President

Labrotur Research and Consultancy Services, LLC
1116 Cavendish Drive, Carmel IN 46032

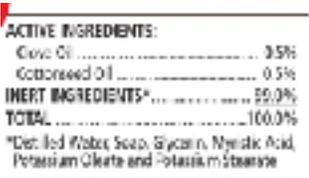


Title: Efficacy Evaluation of Poultry spray® on control of
Alphitobius diaperinus (Darkling beetles) in Turkeys

FACILITY: Turkey Farms
Site: Indiana
Address: 4480 N 100 West
City and State: Washington IN

Confidential

Product Details:

Name of the Product	Poultry Spray Concentrate
Content	 <p>ACTIVE INGREDIENTS: Clove Oil 3.5% Cottonseed Oil 0.5% INERT INGREDIENTS* 96.0% TOTAL 100.0% *Distilled Water, Soap, Glycerin, Myristic Acid, Potassium Chloride and Potassium Stearate</p>
Manufacturer	Davis Manufacturing and Packaging Inc Georgia, USA
Nature of product	Natural parasiticide
Toxicity	nil
Safety	Can be used on animals
Regulatory	Exempt from EPA registration under FIFRA Sec. 25(b)
Recommendations	1 gallon per 1000 sq ft at 1:9 dilution rate (Add 1 gallon of Poultry concentrate to 9 gallons of normal water)

Title: Efficacy Evaluation of Poultry spray® on control of *Alphitobius diaperinus* (Darkling beetles) in Turkeys.

Objective: To demonstrate the efficacy of Poultry Spray on the reduction in darkling beetle population (adults or larvae) in the litter, in Turkeys

Introduction: Darkling beetles (*Alphitobius diaperinus*) are a common problem in poultry facilities. The adults are black with hardened front wings and antennae that start under a ridge near the eyes. The larvae (referred to as lesser mealworms) are worm-like, about 2.5 cm long, and slightly hardened for burrowing. Both the larvae and beetles eat decaying leaves, sticks, grass, dead insects, feces, and grains. They can also feed on dead animals and are known to bite on the skin and drink the blood of turkeys and chickens (Harmon, 2012).

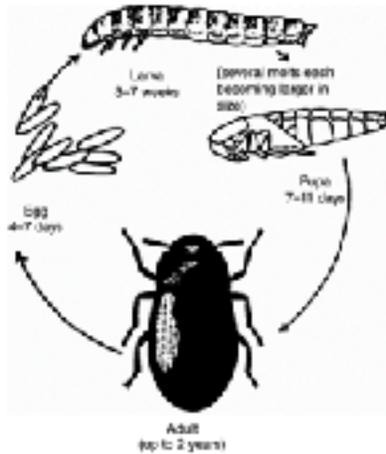
Darkling beetles can carry poultry diseases and internal parasites (Goodwin and Waltman, 1996). The beetles can be a source of infectious laryngotracheitis (Ou et al., 2012), infectious bursal disease (IBD), Newcastle disease, fowl pox, AI, *Clostridium*, *Salmonella* (Roche et al., 2009), *Campylobacter*, and *E. coli*. Darkling beetles are also secondary hosts for roundworms and tapeworms (Adams, 1998). They also host different turkey diseases such as turkey enterovirus, rotavirus, and coronavirus (Harmon, 2012). They are also known reservoirs of *Eimeria*, the causative agent of coccidiosis (Goodwin and Waltman, 1996).

The larvae of darkling beetles can tunnel through insulation and wood, reducing their insulation effectiveness (Vaughan et al., 1984). Chickens will eat darkling beetles, but because of their low digestibility, the birds fill up with them rather than their feed—causing a reduction in production performance.

Several strategies are adopted for the control of darkling beetles in the current industry practices. Industry is looking for better technologies with non-chemical mode of action with better efficacy and cost.

Understanding the Darkling Beetle Lifecycle and its behavior:

Darkling Beetles are one of the most prolific bugs in the poultry houses across the world. Adult Beetles can live up to 2 years if survive all the control measures adopted in the operation. After mating, female start laying eggs within 4-7days. the eggs will hatch and become larvae between 3-7 weeks. Larvae become pupae and remain in that stage for almost 1-2weeks. Finally, Pupae become adult darkling beetles and start laying eggs. Usually, one adult female lay close to 200-400 eggs in 1-5days. Eradication of darkling beetles in the farm premises believe to be practically impossible. The main goal would be to reduce the population as much as possible from one grow to other.



Behavior of Darkling beetles: they appear to be scared of birds, as birds will feed on both adults and larvae. The feeding on darkling beetles increases if there is shortage of feed or longer withdrawal of feed. So, to escape the predation of birds, these darkling beetles hide under the feeder or cakes or burrow underground. These darkling beetles also need abandoned oxygen for their survival. So, the beetles will burrow down only up to the level where they get enough oxygen for their survival. Adults do not like too much moisture or too much temperature. They try to survive in a dry organic matter. Larvae also prefer dry organic matter, but still survive moisture to certain level. Moist litter also deficient in oxygen, so adults try to come to the surface. If the litter is piled up in heap, all the beetles will try to climb the walls or ceiling. Spraying insecticide product during that time will be very effective in killing most of the adults. These beetles are highly prone for resistance development for most classes of compound currently used in the market.

Trial site: In this study, seven brooder farms in Indiana were identified having infestation of darkling beetles (Adults or larvae) in the previous grow out and the details of each farm are provided in the Form no. 1. The following procedure were applied in each farm.

History of farms:

ALBI and II has severe (based on qualitative assessment) adult beetles on the fresh litter and also on the empty floor (Fig 1). All the beetles were seen on the surface. They have severe infestation even in the previous grow out as well.

Cedar Ridge: both the houses were constructed during 2020 and the first flock was placed during August 2020. Since the inception of the farm, the grower never noticed any beetle problems.

Kalb and Bear Hill Farm: both the farms had very severe beetle problems in the previous grow out.

Scenic Hideaway had noticed very mild problems in the past.



Fig 1. ALB I Before arrival of Poults

All the seven farms were following the beetle control program as mentioned below:

1. Boric acid powder on all four sides of the wall – up to 1 foot from the wall. The powder was spread just before spreading the bedding material
2. After spreading the bedding material – Biofen was sprayed through backpack sprayer or through tractor trailer.

Except, Cedar Ridge, all the farms had noticed beetle activities, but unable to interpret the severity of the infestation.

POULTRY SPRAY TREATMENT SCHEDULE:

All the farms have used the fresh litter material obtained from different sources. ALB I, ALB II, Cedar Ridge, Kalb, Bear Hill farms have used rice hulls as the bedding material. Whereas Scenic Hideaway and Loren Graber have used wood shavings as the bedding material.

- ALB I has sprayed the product one time after spreading the bedding material with 1:10 dilution and it was sprayed at the rate of 1 gallon per 1000 sq ft.
- ALB II has sprayed the product on the empty house first and then again one more time after spreading the bedding material.
- Cedar Ridge did one spray after spreading the litter and just before poults arrival.
- Kalb Farm has sprayed the product one time before the poults arrival.
- Bear Hill farm has done three sprays – one on the empty house, one after spreading the bedding material and one after 3 days of poults arrival.
- Scenic Hideaway has done just two sprays with the 24 hours gap before the poults arrival

Confidential

- Loren Graber also has done one spray just before poult's arrival

Darkling Beetles Monitoring Procedure: Monitor visited all the farms on a weekly basis since the arrival of poult. During each visit, monitor checked several spots within the house for beetle activities. The main spots were under the feed lines, water lines, on all sides of the walls, corners, and general areas. In each house and in each examination period, almost 15-20 areas were checked randomly. In each area, approximately, a pound of litter was spread on a large aluminum tray. The beetle activities were observed and estimated the percentage of larvae and adults based on visual observation.

Spray application Procedure: The Poultry spray concentrate was diluted at the rate of 1 gallon of product in 9 gallons of water. The diluted product was sprayed at the rate of 1 gallon per 1000 sq ft. on the litter after spreading the fresh litter in the brooder house.

Observation from field visits:

ALB I

Date	Observations
08/02/2021	Observed severe adult beetles on the surface of the litter. Fresh litter was spread on 7/27/2021. No other spray or powder insecticide product was applied in this house; based on the severity of beetle population, two sprays were advised with in the gap of 24 hours. NO spray was done before spreading the litter material.
08/10/2021	Very few live beetles were noticed in the litter, especially under the papers. But most of the beetles are appeared to be dead as there were too many dead one observed on the litter. Since it is a fresh litter, those dead beetles must be from the spray done before the poult's arrival.
08/20/2021	Mild to moderate beetle (both adults and larvae) activities were observed under feeders in first 1/3rd of the house. Mild activities were noticed in the second 1/3rd of the house. there were no activities in the last 1/3rd of the house. There were no beetles found in other areas. around the feeders, there was cake formation, and the beetles were noticed under those cakes.
08/24/2021	Severe beetle activities in first 1/3rd of the house – mainly under feeders. Moderate activities in the second 1/3rd of the house, mild activities in the last 1/3rd of the house were observed. Mild beetle activities in the general areas, especially in first 1/3rd of the house. Not much in the second and last 1/3rd of the house.

Confidential

08/31/2021	No change in status compared to previous week. Only difference was, all the beetles (both larvae and adults) were seen under the cakes only. No beetles were seen in the dry and free flowing litter. The larvae was almost 60% and adults were around 40% (percentage estimates are based on visual observations only - qualitative)
09/07/2021	Severe beetle activity were observed in most of the areas. The activity was seen only under the cakes. 95% of them were larvae and around 5% were adults which was analyzed using Aluminum tray method. During previous visit, the judgement was done based on visual observation. but this time, the litter sample was placed on the aluminum tray and physically counted the adult population which was very low in number.
09/13/2021	Birds were moved to Grower house on 9/10/21. on the same day, the litter was removed and trashed. Today, lot of adult beetles were seen on the floor, side walls and ceiling.

ALB II

Date	Observations
08/02/2021	Lot of beetles were observed on the empty concrete floor. The litter was spread on 08/03/2021. One spray was done on the empty floor to kill all the live adults.
08/10/2021	No adult live beetles were observed in the litter on this day.
08/20/2021	Mild beetle (both adults and larvae) activities noticed under feeders in first 1/3rd of the house. There were no activities in the rest of the house. there were no beetles found in general areas.
08/24/2021	Moderate beetle activities in the first 1/3rd of the house, especially under feeders. Mild activities in the second 1/3rd and nil activities as well. In the last 1/3rd of the house. No beetle activities in general areas.
08/31/2021	In this house also, beetles are seen under the cakes only. The percentage of adults are seeming to be around 20%. Again overall severity of beetle population is much lower than ALB I. (percentage estimates are based on visual observations only - qualitative)
09/07/2021	Moderate beetle activity with more than 98% larvae.
09/13/2021	Birds were moved to grower house on this day. Beetle activities were noticed only under feeders, especially under cakes. Very mild activities in the sides. There were no beetle activities in other area. Wherever the beetle activities were seen, there was more than 95% larvae and 5% adults.

Confidential

Cedar Ridge

Date	Observations
08/10/2021	The farm is a new construction and started in August 2020. They have not experienced beetle problems since the beginning of this facility. Even today, no beetles were observed.
08/20/2021	No Beetle activities found in any of the two houses
08/24/2021	No Beetle activities found in any of the two houses
08/31/2021	No Beetle activities found in any of the two houses
09/07/2021	No Beetle activities found in any of the two houses
09/13/2021	Birds were moved to Grower house on 9/08/21 from house # 1. birds were moved on 09/09/21 from house # 2. NO beetle activities noticed in both the houses.

KALB FARM

Date	Observations
08/10/2021	Few adult live beetles were observed today especially on the corners. But lot of dead beetles were observed in the litter. No other treatment in terms of spray or powder were applied before spreading the litter.
08/20/2021	No Beetle activities found in any part of the house.
08/24/2021	No beetle activities almost 90% of the house, except mild to moderate activities only on the south side of the entire length of the house - just one feed from the wall. Whereas no beetle activities on the North side of the house. Flies were noticed inside the barn.
08/31/2021	Mild beetle activities were seen on both sides of the wall. No beetle activities in any other area of the house. 80% larvae and 20% adults were noticed in the samples. (Percentage estimates are based on visual observations only - qualitative)
09/07/2021	Aluminum tray method was used to quantify the adults and larvae and accordingly, the observation was that the larvae is almost 95% and 5% of adults. Again, the beetle activity was seen only 1/3rd of both the sides from the wall. There was no

Confidential

09/13/2021	Birds were moved to Grower house on this day. Beetle activities were seen everywhere in the house, but 98% are larvae. Grower is not interested in spraying our product in the coming flock.
------------	----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

Bear Hill Farm

Date	Observations
08/10/2021	Previous brooder grow out had very severe darkling beetles problems. Grower saw the beetles climbing walls and ceiling. In this grow out, the litter is already spread. The heating will start on 08/12/2021 morning and the beetles are expected to come down. the spray will be done on 08/12 afternoon and 08/13 morning. poults are expected in the later afternoon on 08/13
08/13/2021	one spray was done on the empty floor just before spreading the litter. After spreading the litter, one more spray was done. The severe beetle activities were noticed during the spray of the product. 24 hours after poults arrival, adult beetles were noticed under dead birds.
08/20/2021	One more spray was done on this day. But no adult beetles were noticed in any part of the house. lot of dead ones were observed in the litter. The beetles would have either dead completely or some of the surviving one might have left the house due to excessive heat during the brooding period.
08/24/2021	No beetle activities in almost 98% if the house, except very mild adult and larvae beetles were found in the house entrance.
08/31/2021	Mild beetle activities are seen on both sides of the wall. No beetle activities in any other area of the house. 80% larvae and 20% adults were noticed in the samples. (percentage estimates are based on visual observations only - qualitative)
09/07/2021	Aluminum tray method was used to quantify the adults and larvae and accordingly, the observation was that, the larvae is almost 98% and 2% of adults. Again, the beetle activity was seen only in 1/3rd of both the sides from the wall. There was no activity in the center of the house.
09/13/2021	Birds will be moved to grower house on 09/17/21. But could not visit the house during this time.

Scenic Hideaway Farm

Date	Observations
-------------	---------------------

Confidential

08/10/2021	11-year-old farm. Few beetles were seen before and during spray on the surface of the litter. No live beetles were observed on this day.
08/20/2021	very few adult beetles were noticed on one side of the house. there were no beetle activities under the feeders or any other part of the house.
08/24/2021	Mild beetle activities in the north side (first 1/3rd only) of the house (only in certain pockets) - only 1/2 feet from the wall. Very mild activities in the south side, again only in certain pockets, especially near the pillars. No activities under feeders or general areas.
08/31/2021	Same as previous week observation. No major changes.
09/07/2021	Mild beetle activities in few spots, especially in the sides. 95% of them are larvae. Birds are getting moved on 09/08. later, the litter will be piled up in heap and moved on 09/09.
09/13/2021	visited the farm on 09/09. very few adults were noticed on the pile of litter, lot of larvae was noticed in the crevices and under the litter pile. The litter was moved to grower house on 09/10.

Loren Graber Farm

Date	Observations
08/10/2021	B on the grower's feedback, few live adult beetles were seeing before the spray. Today no beetles were seen in any part of the house. no other treatment is applied as spray or powder.
08/20/2021	No beetle activities noticed in any part of the house.
08/24/2021	No beetle activities.
08/31/2021	No beetle activities.
09/07/2021	did not visit the farm as there was no beetle activity reported before
09/13/2021	Very mild beetle activities, only in few spots, in those spots, the larvae are almost 90% and less than 10% is the adults. Birds will be moved to the grower house on 09/17/2021.

Summary and Discussion

Darkling Beetles are always a serious concern for the producers as it damages the structure, transmit many diseases, affects bird performance etc., Producers/integrated companies are adopting various programs on a rotation basis. Resistance development for various chemicals, pyrethrin, pyrethroids, borates, organo-phosphorus by the adults and larvae is very common.

Confidential

Any product with physical mode of action and lesser chances of resistance development are preferred to mitigate the beetle population. Eradication is practically impossible, but the population can be kept under control while ensuring better performance of the flock.

Both adults and larvae do need oxygen for their survival and at the same time, they try to hide from the birds. They try to hide under litter, cakes, feeders, dead bird, or any other covering which prevents the direct access with the birds. Also, beetles prefer below the brooding temperature and lower moisture. During the windrowing process, most of the larvae gets killed due to high temperature and moisture.

Usually, just before the new flock arrival, all the adult beetles come down from the side walls or ceiling as they realize the absence of birds and nice warm climate just above the litter surface. Most of the adult beetles are seen huddling on the surface during this period and can be a good target for killing them. During this period, adults may be involved in mating or laying eggs. Those female adults may lay eggs before killed by the insecticide products or those female adults which escape the action of insecticide product may lay eggs during first week of the brooding life. These beetle eggs will hatch during 2-3 weeks of poults/chicks brooding period. One adult female beetle may lay up to 400 eggs in 1-5 days after mating. So, it is very important to kill all these adults as much as possible before the bird's arrival. For those products which do not have residual effect may be used for 1-3 times before the bird's arrival. Again, one or two sprays are preferred after the grow out and just before the adult beetles climb up the walls and ceiling. Ideally it is recommended to windrow the litter after each grow out flock, so that most of the adults migrate to the surface and all the larvae die due to excessive temperature and humidity. Spraying an insecticide on the surface of the windrow pile of litter would kill most of the beetles. Such practices would help in reducing the population over a period of time.

Conclusion:

'Davis Poultry Spray' product application has shown a definite reduction in adult live beetle population at the end when compared to the beginning of the grow out. But the heavy larvae presence misguides the efficacy of the product. The product was very effective in killing all the adults which came in contact with the spray. The higher larvae population was because of either adult beetles would have laid eggs before dying or the ones who survived the spray would have laid eggs in the later part. Generally, larvae are harmless in turkey production as they feed only on the decaying organic matter or feces and also, the used litter will be trashed after each grow out. Adult beetle population control is critical as they have a tendency to stay in the facility for long period of time.

In the future, spraying 'Davis Poultry Spray' product one to three times before the chicks or poults arrival will be very helpful in reducing most of the adult population. The number of spray application should be decided by the grower and the company representative

based on the severity of infestation. Also, measuring number of adults per pound of litter at different spots in the house before placement and after transfer of poults (in cause of turkeys) or after marketing of birds (in case of broilers) will help us in understanding the efficacy of the product. In case of large bird broiler operation where windrowing is not performed, there is a possibility of new adult beetle formation from the underlaying pupae. So, one has to be extra cautious in interpreting the efficacy data in in broilers.

Form 1 – Farms Enrolled in the study

Name and Address of the Farm	Capacity of the brooder	Poults input date	Date of first spray	Study end date
ALB I LOGAN GRABER 1490 S 700 E MONTGOMERY IN 47558 PHONE - 812 787 0587	12,500 sq ft – 20,030 poults	08/03/2021	08/02-03/2021 – two sprays	09/08/2021
ALB II LOGAN GRABER 1490 S 700 E MONTGOMERY IN 47558 PHONE - 812 787 0587	12,500 SQ FT – 19,198 poults	08/06/2021	08/03/2021 and 08/06/2021 – two sprays	09/13/2021
CEDAR RIDGE KEITH KNEPP 3656 E 225 S WASHINGTON IN	22,500 SQ FT – TWO HOUSES – STARTED Aug 2020	08/06/2021 House I and 8/10/2021 House II	08/06/2021 - House I and 08/10-12/2021 - house II	09/08-10/2021
KALB FARM 6404 N 445 E DUBOIS IN 47527 Erin Kalb	10,000SQ FT	08/06/2021	08/05/2021 – one spray before poult arrival	09/13/2021
BEAR HILL 6404 N 445 E DUBOIS IN 47527 Erin Kalb	10,000SQ FT	08/13/2021	08/13-16/2021 – THREE SPRAYS	09/17/2021

Confidential

Form 1 – Farms Enrolled in the study

Name and Address of the Farm	Capacity of the brooder	Poults input date	Date of first spray	Study end date
SCENIC HIDEAWAY 12255 ZEHR LN LOGOOTE 47501 HAROLD YODER	10,000 sq ft	08/06/2021	08/02/2021 AND 08/03/2021	09/09/2021
LOREN GRABER 7134 N 1000 W MONTGOMERY IN 47553	16,000 SQ FT	08/05/2021	08/03/2021	09/17/2021