COMPARATIVE TOXICITY OF FLUBENDIAMIDE AND LUFENURON INSECTICIDES ON *TRIBOLIUM CASTENIUM* UNDER LABORATORY CONDITIONS

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ABSTRACT

Toxicity of insecticides *viz*. lufenuron 5 EC; flubendiamide 480 SC respectively was tested on different larval stages of *Tribolium castenium* in lab. In this study , the concentrations of flubendiamide and lufenuron were 5, 10, and 20 µg/ml in Petri dish with 12 sets of treatment and 3 replication of each. Lufenuron and flubendiamide on the various developmental stages of red flour beetle *Tribolium castenium* was determined by exposing them to different sub- lethal concentration (LC_5 , LC_{10}, LC_{20}, LC_{40}) of flubendiamide through diet for 12 hrs, 24 hrs, 48hrs respectively. The observations on mortality of different stages of *T. castenium* larvae were recorded after 3, 6, 12, 24 and 48 hours of application. The application of lufenuron proved to be comparatively safer with 8 to 16 percent mortality of different larval stage of *T.castenium* at all intervals. Flubendiamide was found as intermediately toxic in all post treatment intervals. Hatching capacity of flubendiamide and even at sub-lethal concentration shows good larvicidal and ovidicidal activity in case of lufenuron also larvicidal and ovicidal activity was good.

KEYWORDS: flubendiamide, lufenuron, *Tribolium castenium*, insecticide.

INTRODUCTION

Pesticides are the only toxic substances released intentionally into our environment to kill living things. This includes substances that kill weeds (herbicides), insects (insecticides), fungus (fungicides), rodents (rodenticides), and others. The word pesticides is composed term including any substances or mixture of substances intended for preventing , destroying , repelling or mitigating any pest (rigert et.al 1999).pesticides are often referred to according to the type of pest they control . Another way to think about pesticide is to consider those that are chemical pesticides are or derived from a common source or production method.

Insecticides which are specific and with novel mode of action such as flubendiamide and lufenuron not only help to control the pest population but also helps in maintaining a balance in biodiversity. Being specific on pest action some insecticides can effect these beneficial insects. Application of selective insecticides to control pests could be useful in conservation of natural enemies associated

with crops. Some insecticides are potentially more toxic to the target pest but not to natural enemies, thus playing significant role in conservation of biological control agents in agricultural environments. Flubendiamide have a novel mode of action, because the insecticidal symptoms accompanied by a discriminative contraction of the larval body are distinguished from those of commercial insecticides. It is also very safe for non-target organisms. Flubendiamide is expected to be a suitable agent for controlling insects as part of the insect resistance management and the integrated pest management programs.

Insecticidal activity through the activation of the ryanodine-sensitive intracellular calcium release channels, leading to the cessation of feeding immediately after ingestion of the compound, this leads to shrinking and stiffening f the larval body. They do not affect mammalian ryanodine receptors, most probably explaining their excellent toxicological profile, being specific to the pests and not affecting natural predators or pollinators and being non-toxic to mammals, fishes and birds.

MATERIALS AND METHOD: A stock culture of *T. castaneum* was maintained on a diet containing

wheat flour, at room temperature.


Eggs were collected by sieving (sieve number 40) diet infested with adults. Newly emerged adults were obtained by collecting pupae and monitoring them for adult emergence. Solution of lufenuron and flubendiamide was prepared having, the concentrations 5, 10, 20 and 40 µg/ml in Petri dish with 12 treatment and 3 replication of each. Different volumes of lufenuron and flubendiamide were thoroughly incorporated into diet. The treated flour was kept at room temperature for twenty four hours, for complete evaporation of the solvent before use in the experiments. Acetone mixed diet was used as control. The control and experimental units were kept in a cooling incubator at 30˚C before and after the treatment. For each concentration tested sets of five replicates of twenty larvae each were taken. The mortality count was taken after seven days. Effect concentration of lufenuron and flubendiamide through diet on survival and metamorphosis of the larvae was examined by releasing two day old larvae (20 larvae per replicate and three replicates per treatment) in the treated diet. After 24 hours, the larvae were transferred to normal diet. Once pupation had begun in any treatment, observations were made every day for adult emergence. Pupation, time taken for pupation, adult emergence and time taken for adult emergence were recorded. The mortality counts were deduced by extrapolation from the regression analysis.

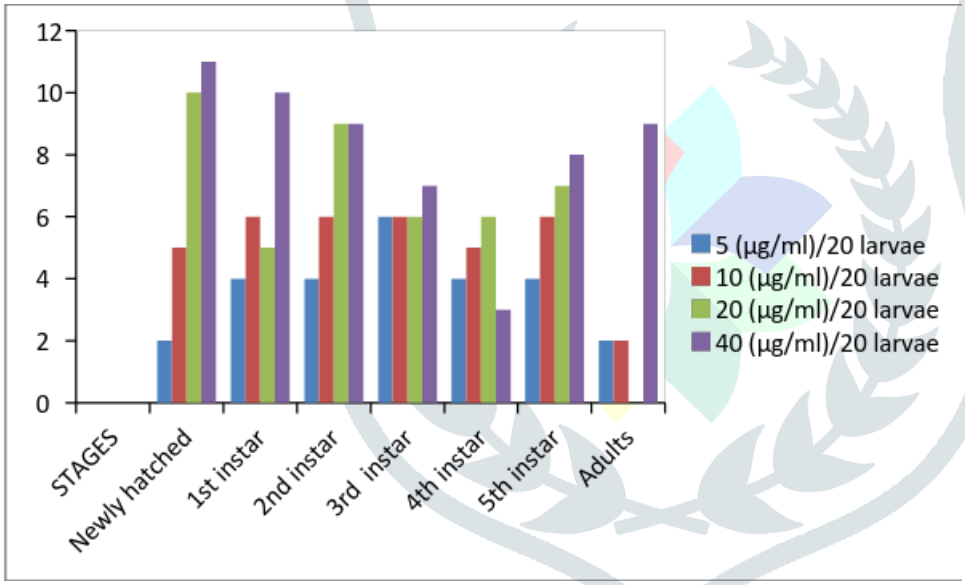
RESULT AND DISCUSSION:

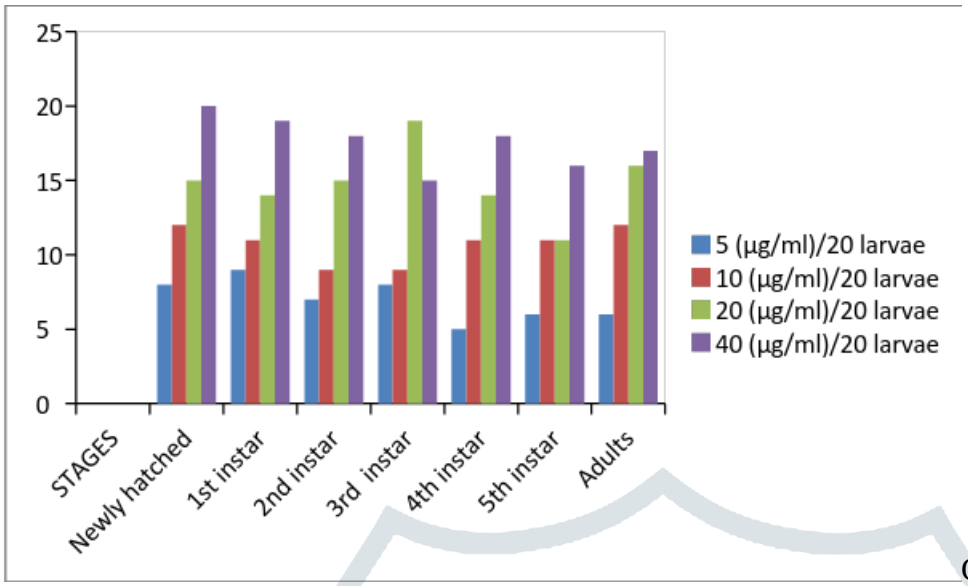
Data obtained from flubendiamide were as follows

Flubendiamide – 12 hour of application (table -1)

CONCENTRATION	5 (µg/ml)/20 larvae	10 (µg/ml)/20 larvae	20 (µg/ml)/20 larvae	40 (µg/ml)/20 larvae
STAGES				
Newly hatched	02	05	10	11
1st instar	04	06	05	10
2nd instar	04	06	09	09
3rd instar	06	06	06	07
4th instar	04	05	06	03
5th instar	04	06	07	08
Adults	02	02	00	09

Graph-1





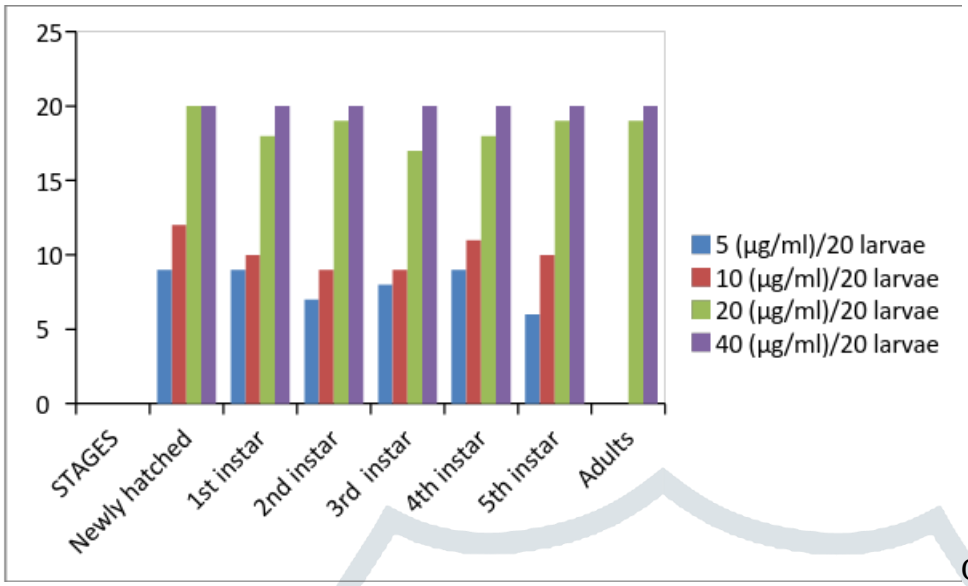
Graph-2

FLUBENDIAMIDE- 24HOURS (table -2)

CONCENTRATION	5 (µg/ml)/20 larvae	10 (µg/ml)/20 larvae	20 (µg/ml)/20 larvae	40 (µg/ml)/20 larvae
STAGES				
Newly hatched	08	12	17	20
1st instar	09	11	18	19
2nd instar	07	09	17	18
3rd instar	08	09	19	15
4th instar	05	11	19	18
5th instar	06	11	18	16

FLUBENDIAMIDE- 48HOURS (table -3)

CONCENTRATION	5 (µg/ml)/20 larvae	10 (µg/ml)/20 larvae	20 (µg/ml)/20 larvae	40 (µg/ml)/20 larvae
STAGES				
Newly hatched	09	12	20	20
1st instar	09	10	18	20
2nd instar	07	09	19	20
3rd instar	08	09	17	20
4th instar	09	11	18	20
5th instar	06	10	19	20
Adults			19	20



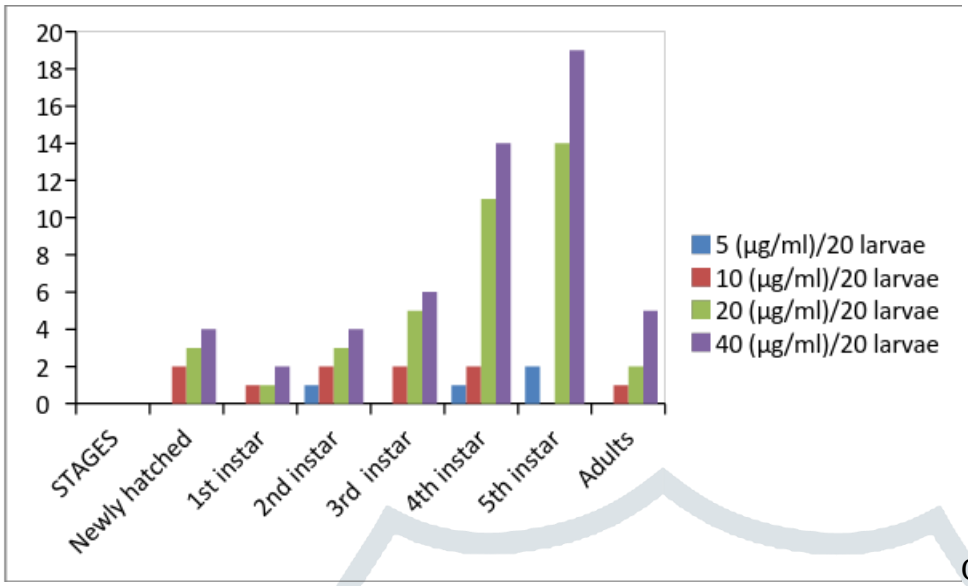
Graph-3

From the graph above (table no -1, 2 , 3) it's been concluded that as the duration and concentration increases the mortality also increases. Graph no 1 show within 12 hour highest mortality is seen in 40 micro liters per ml. from table graph no 2 even in 20 (µg/ml) more than 70 percent mortality is seen this proves that duration also increases the rate of mortality. In 48 hour of application in high concentration the mortality is almost hundred percent, although the mortality in 5 and 10 (µg/ml) is low but it has increased with duration.

LUFENURON

LUFENURON- 48 HOUR (TABLE-4)

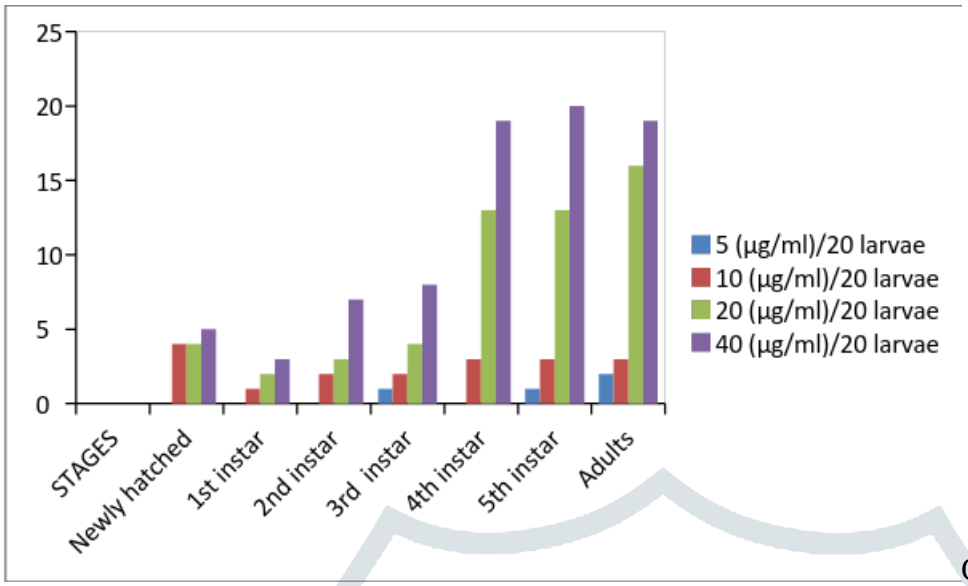
CONCENTRATION	5 (µg/ml)/20 larvae	10 (µg/ml)/20 larvae	20 (µg/ml)/20 larvae	40 (µg/ml)/20 larvae
STAGES				
Newly hatched	00	02	03	04
1st instar	00	01	01	02
2nd instar	01	02	03	04
3rd instar	00	02	05	06
4th instar	01	02	11	14
5th instar	02	00	14	19
Adults	00	01	02	05



Graph-4

LUFENURON- 72 HOUR (table-5)

CONCENTRATION	5 (µg/ml)/20 larvae	10 (µg/ml)/20 larvae	20 (µg/ml)/20 larvae	40 (µg/ml)/20 larvae
STAGES				
Newly hatched	00	04	04	05
1st instar	00	01	02	03
2nd instar	00	02	03	07
3rd instar	01	02	04	08
4th instar	00	03	13	19
5th instar	01	03	13	20
Adults	02	03	16	19

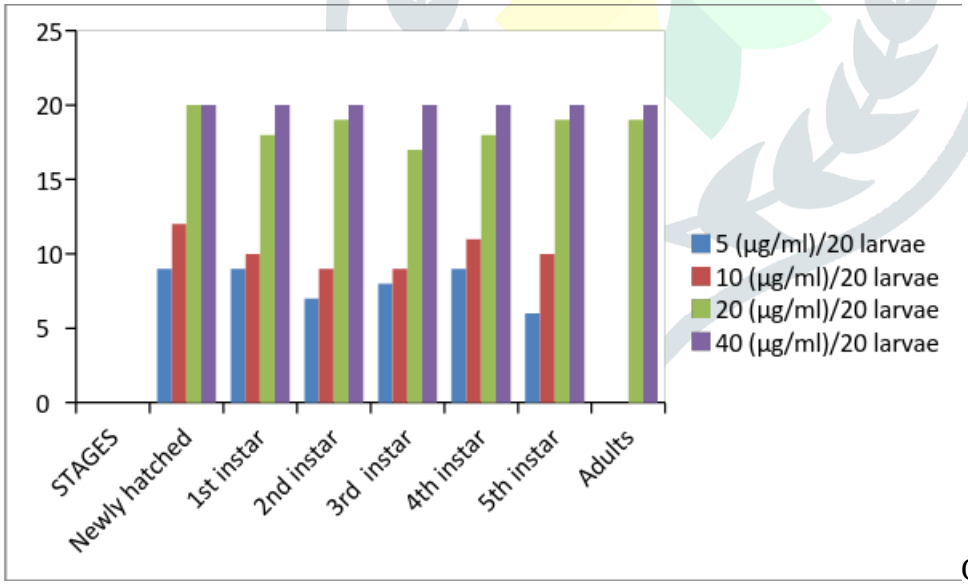


Graph 5

Lufenuron does not have effective mortality on 1st, newly hatched larvae, 2nd and on 3rd larvae on less duration .in 48 hours of duration on 4th and 5th instars of larvae larvae-pupae intermediated were seen.

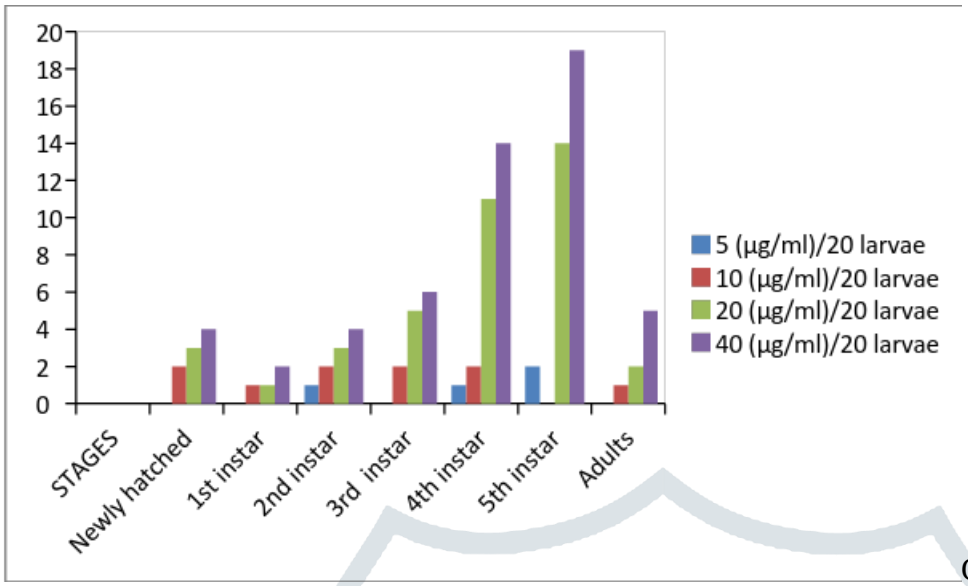
Comparison of lufenuron and flubendiamide insecticides at 48 hour of duration on different instars of larvae

FLUBENDIAMIDE -48 HR (table – 6)



Graph 6

LUFENURON -48 HR (TABLE- 7)



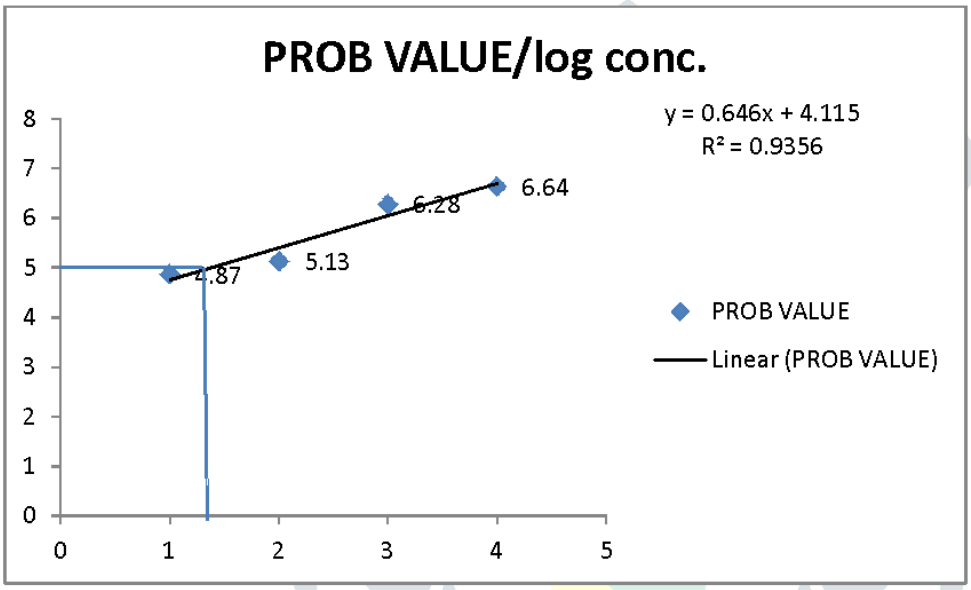
Graph 7

This shows that in Graph-6 mortality is positive in every stage but in Graph-7 the mortality is positive only regarding 4th and 5th instar of larvae and not in adults.

PROBIT REGRESSION ANALYSIS

LC-50 FOR 24 HOUR IN CASE OF FLUBENDIAMIDE FOR 1 st INSTAR LARVAE

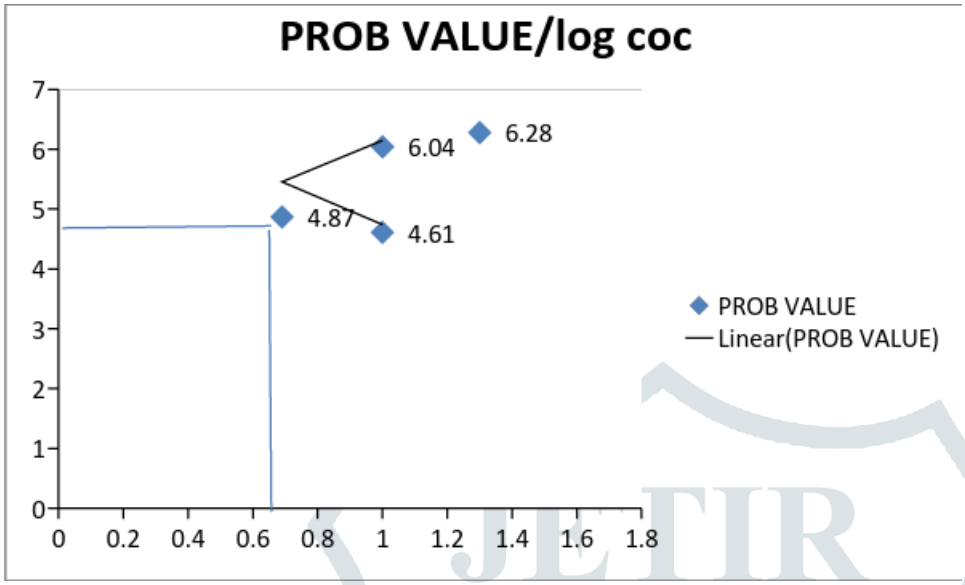
SR.NO	TOXICANT CONCENTRATION	CONC. OF TOXICANT (PPM)	LOG CONC OF TOXICANT	TOTAL NO.	NO. OF DEAD INSECTS	PERCENT MORTALITY	PROB VALUE
1	5 µg/ml	5	0.69	20	9	45	4.87
2	10 µg/ml	10	1	20	11	55	5.13
3	20 µg/ml	20	1.30	20	18	90	6.28
4	40 µg/ml	40	1.60	20	19	95	6.64



LC50 for 24 hours of pesticides application is 1.4 µg/ml on 1st instar larvae.

LC-50 FOR 24 HOUR IN CASE OF FLUBENDIAMIDE FOR 2 nd INSTAR LARVAE

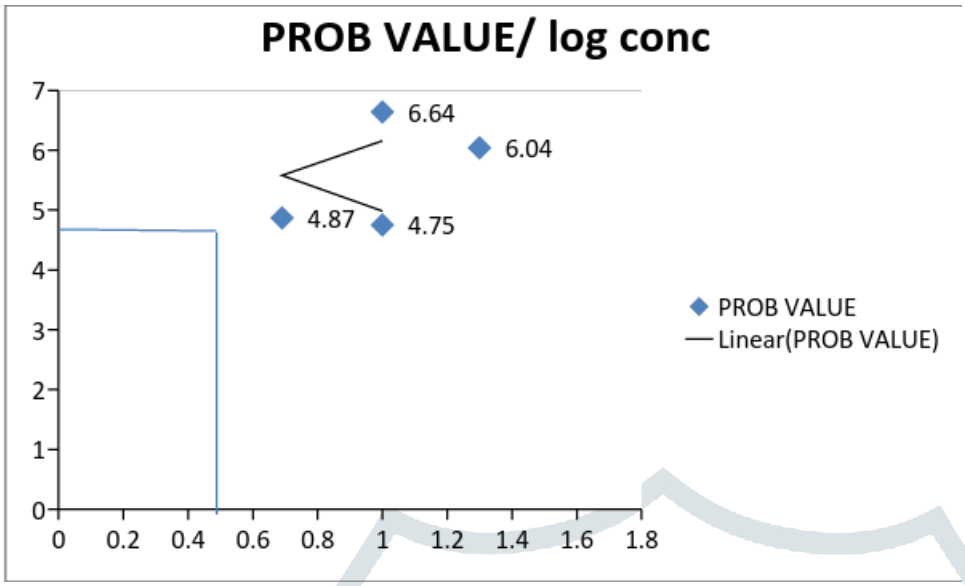
SR.NO	TOXICANT CONCENTRATION	CONC. OF TOXICANT (PPM)	LOG CONC OF TOXICANT	TOTAL NO.	NO. OF DEAD INSECTS	PERCENT MORTALITY	PROB VALUE
1	5 µg/ml	5	0.69	20	7	35	4.61
2	10 µg/ml	10	1	20	9	45	4.87
3	20 µg/ml	20	1.30	20	17	85	6.04
4	40 µg/ml	40	1.60	20	18	90	6.28



LC50 for 24 hour of application of flubendiamide if found to be 1.9 µg/ml on 2nd instar larvae.

LC-50 FOR 24 HOUR IN CASE OF FLUBENDIAMIDE FOR 3rd INSTAR LARVAE

SR.NO	TOXICANT CONCENTRATION	CONC. OF TOXICANT (PPM)	LOG CONC OF TOXICANT	TOTAL NO.	NO. OF DEAD INSECTS	PERCENT MORTALITY	PROB VALUE
1	5 µg/ml	5	0.69	20	8	40	4.75
2	10 µg/ml	10	1	20	9	45	4.87
3	20 µg/ml	20	1.30	20	19	95	6.64
4	40 µg/ml	40	1.60	20	17	85	6.04

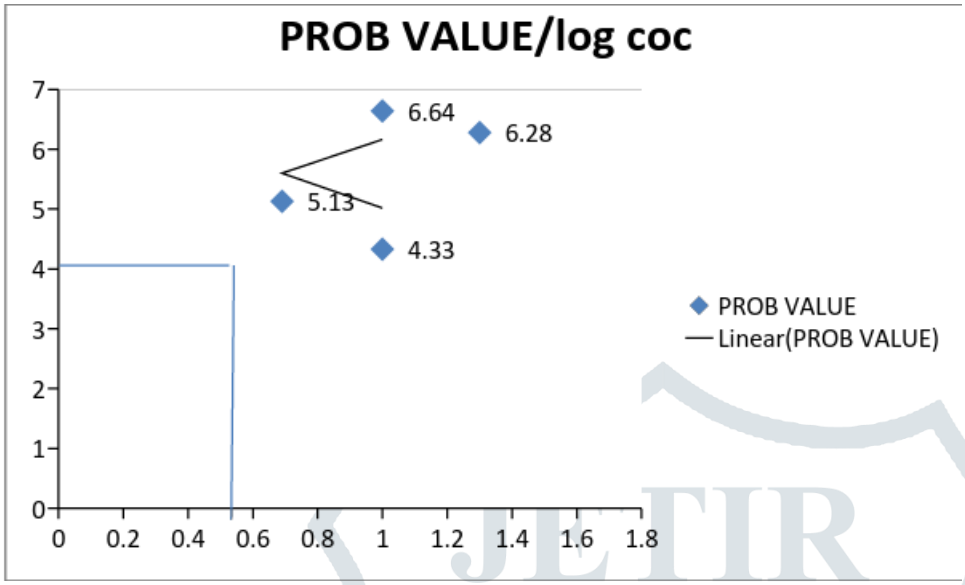


LC50 for flubendiamide for 24 hour of application is found to be 1.5 µg/ml on 3rd instar larvae.

LC-50 FOR 24 HOUR IN CASE OF FLUBENDIAMIDE FOR 4th INSTAR LARVAE

SR.NO	TOXICANT CONCENTRATION	CONC. OF TOXICANT (PPM)	LOG CONC OF TOXICANT	TOTAL NO.	NO. OF DEAD INSECTS	PERCENT MORTALITY	PROB VALUE
1	5 µg/ml	5	0.69	20	5	25	4.33
2	10 µg/ml	10	1	20	11	55	5.13
3	20 µg/ml	20	1.30	20	19	95	6.64
4	40 µg/ml	40	1.60	20	18	90	6.28

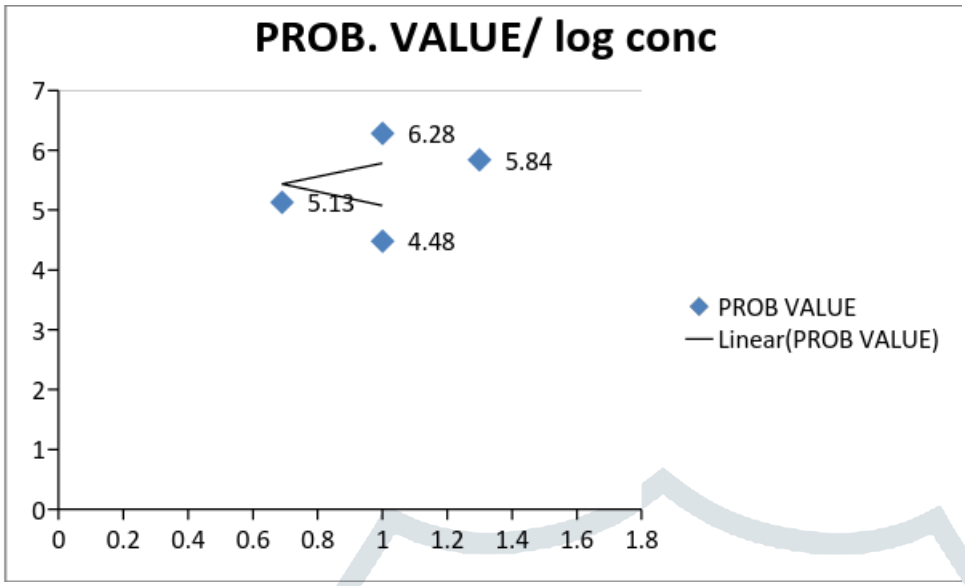
SR.NO	TOXICANT CONCENTRATION	CONC. OF TOXICANT (PPM)	LOG CONC OF TOXICANT	TOTAL NO.	NO. OF DEAD INSECTS	PERCENT MORTALITY	PROB VALUE
1	5 µg/ml	5	0.69	20	6	30	4.48
2	10 µg/ml	10	1	20	11	55	5.13
3	20 µg/ml	20	1.30	20	18	90	6.28
4	40 µg/ml	40	1.60	20	16	80	5.84



LC 50 FOR 24 hour of flubendiamide application is found to be 1.5 µg/ml 4th instar larvae

LC-50 FOR 24 HOUR IN CASE OF FLUBENDIAMIDE FOR 5th INSTAR LARVAE

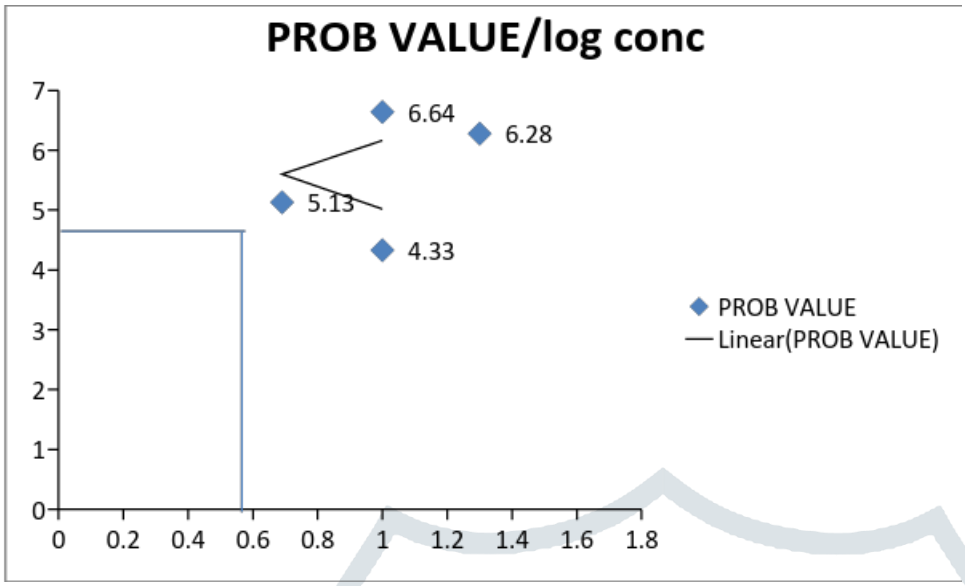
SR.NO	TOXICANT CONCENTRATION	CONC. OF TOXICANT (PPM)	LOG CONC OF TOXICANT	TOTAL NO.	NO. OF DEAD INSECTS	PERCENT MORTALITY	PROB VALUE
1	5 µg/ml	5	0.69	20	6	30	4.48
2	10 µg/ml	10	1	20	11	55	5.13
3	20 µg/ml	20	1.30	20	18	90	6.28
4	40 µg/ml	40	1.60	20	16	80	5.84



LC50 for 24 hour of application of flubendiamide is found to be 1.9 µg/ml 5th instar larvae.

LC-50 FOR 24 HOUR IN CASE OF FLUBENDIAMIDE FOR ADULT

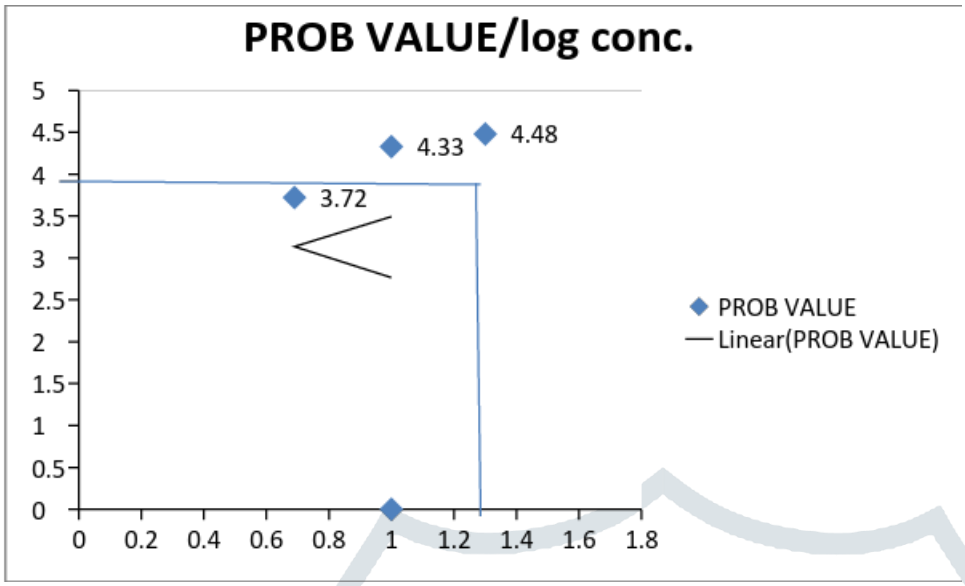
SR.NO	TOXICANT CONCENTRATION	CONC. OF TOXICANT (PPM)	LOG CONC OF TOXICANT	TOTAL NO.	NO. OF DEAD INSECTS	PERCENT MORTALITY	PROB VALUE
1	5 µg/ml	5	0.69	20	6	30	4.48
2	10 µg/ml	10	1	20	12	60	5.25
3	20 µg/ml	20	1.30	20	16	80	5.84
4	40 µg/ml	40	1.60	20	17	85	6.04



LC50 FOR flubendiamide for 24 hour of application is found to be 1.7 µg/ml on adults.

LC-50 FOR 48 HOUR FOR LUFENURON ON 3rd INSTAR LARVAE

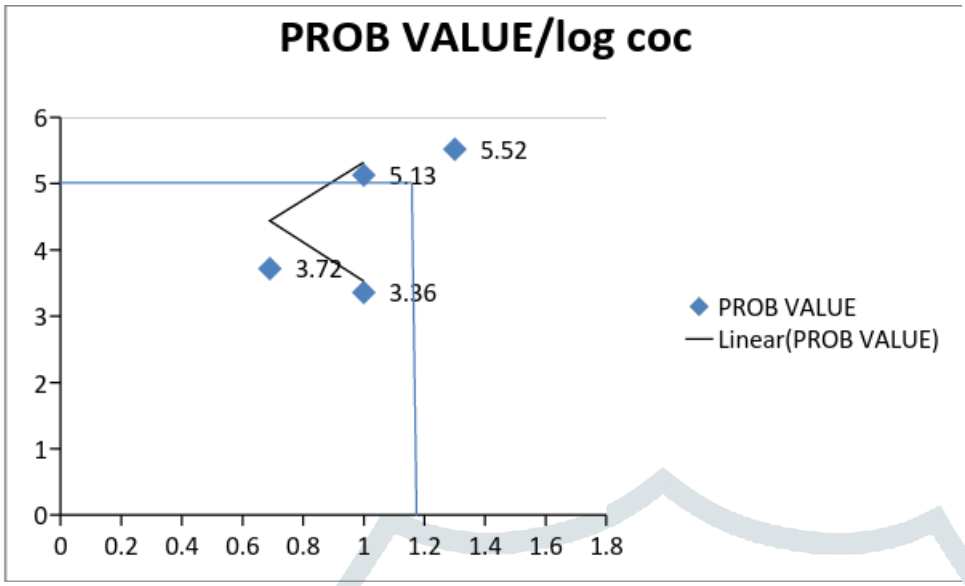
SR.NO	TOXICANT CONCENTRATION	CONC. OF TOXICANT (PPM)	LOG CONC OF TOXICANT	TOTAL NO.	NO. OF DEAD INSECTS	PERCENT MORTALITY	PROB VALUE
1	5 µg/ml	5	0.69	20	0	0	0
2	10 µg/ml	10	1	20	2	10	3.72
3	20 µg/ml	20	1.30	20	5	25	4.33
4	40 µg/ml	40	1.60	20	6	30	4.48



LC 50 for lufenuron for 48 hour of application is found to be 3.9 µg/ml on 3rd instar larvae.

LC-50 FOR 48 HOUR FOR LUFENURON ON 4TH INSTAR LARVAE

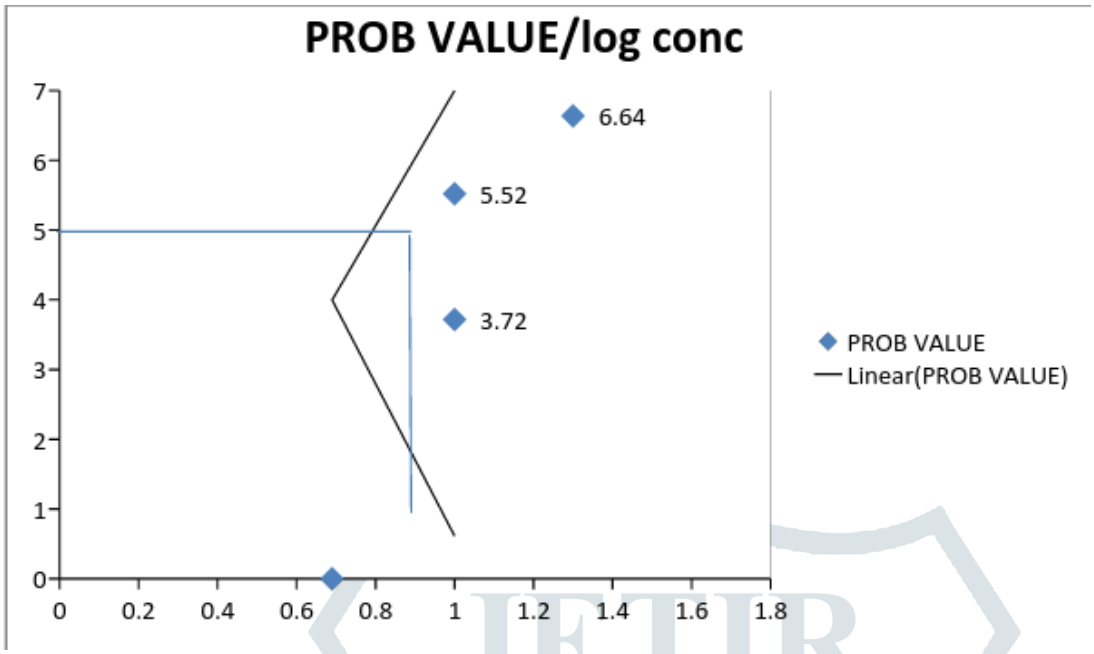
SR.NO	TOXICANT CONCENTRATION	CONC. OF TOXICANT (PPM)	LOG CONC OF TOXICANT	TOTAL NO.	NO. OF DEAD INSECTS	PERCENT MORTALITY	PROB VALUE
1	5 µg/ml	5	0.69	20	1	5	3.36
2	10 µg/ml	10	1	20	2	10	3.72
3	20 µg/ml	20	1.30	20	11	55	5.13
4	40 µg/ml	40	1.60	20	14	70	5.52



LC 50 for 48 hour of lufenuron application id found to be 3.2 µg/ml on 4th instar larvae.

LC-50 FOR 48 HOUR IN CASE OF LUFENURON FOR 5th INSTAR LARVAE

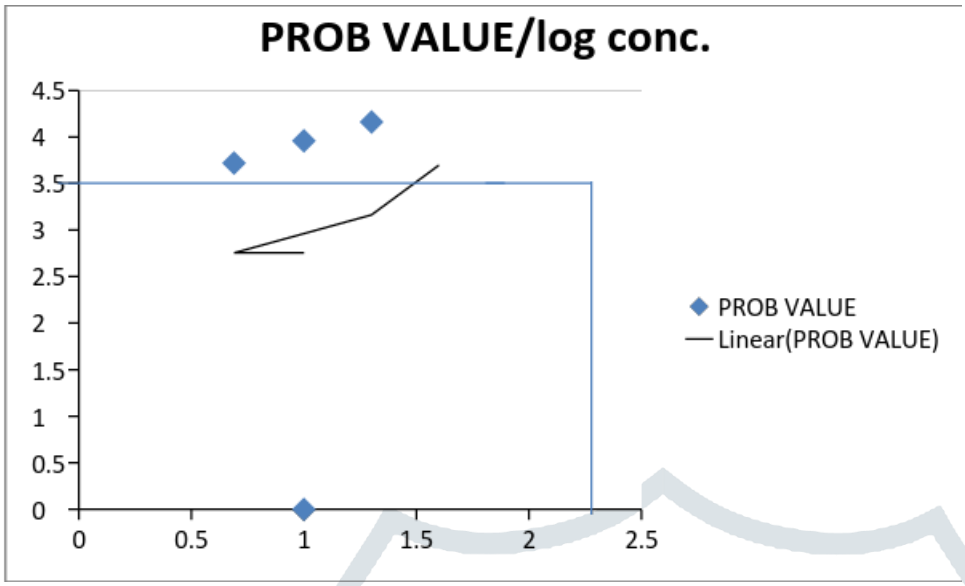
SR.NO	TOXICANT CONCENTRATION	CONC. OF TOXICANT (PPM)	LOG CONC OF TOXICANT	TOTAL NO.	NO. OF DEAD INSECTS	PERCENT MORTALITY	PROB VALUE
1	5 µg/ml	5	0.69	20	2	10	3.72
2	10 µg/ml	10	1	20	0	0	0
3	20 µg/ml	20	1.30	20	14	70	5.52
4	40 µg/ml	40	1.60	20	19	95	6.64



LC50 for 48 hour of lufenuron application is found to be 3.2 µg/ml on 5th instar larvae.

LC-50 FOR 48 HOUR IN CASE OF LUFENURON FOR NEWLY HATCHED LARVAE

SR.NO	TOXICANT CONCENTRATION	CONC. OF TOXICANT (PPM)	LOG CONC OF TOXICANT	TOTAL NO.	NO. OF DEAD INSECTS	PERCENT MORTALITY	PROB VALUE
1	5 µg/ml	5	0.69	20	0	0	0
2	10 µg/ml	10	1	20	2	10	3.72
3	20 µg/ml	20	1.30	20	3	15	3.96
4	40 µg/ml	40	1.60	20	4	20	4.16



LC 50 for 48 hour of lufenuron application is found to be 4.9 µg/ml on newly hatched larvae.

In case of lufenuron significant increase in time taken for pupation and adult emergence were observed. Lufenuron treated 1st and 2nd instar larvae could not molt. In 3rd instar, high pupal mortality occurred.. A significant decrease in percent pupation and percent adult emergence were observed in a dose-dependent manner. At concentration of and 40 µg/ml in 5th stage larvae molting of pupa into adult was affected, resulting in the development of pupal-adult intermediates. Further, it was found that, number of eggs laid by adults developed from larvae treated lufenuron was not significantly different from that of control. Minimum effects were seen on 1st, 2nd and 3rd stage larvae. Also, percent hatching of such eggs and percent survival of the larvae was not affected it also does not show lethal affect on adults. The application of flubendiamide at 1st 2nd and 3rd instars of *T.castenium* larvae shows minimum mortality. after 3,6,12 and 24 hours in minimum concentration were 5,and 10 µg/ml Highest mortality is seen in both 4th and 5th stage larvae where the concentration was 20 µg/ml within 48 hrs of post treatment. Body of larvae shrinks and becomes half of the size cessation of movement is seen in all stars of larvae after post treatment. Hatching of treated eggs were not affected. Hence it's concluded that lufenuron affects the molting process and results in intermediate larvae in 5th star larvae, while flubendiamide kills the larvae within 72 hours of treatment. it Cassese's the movement of larvae in low concentration and kills the larvae in higher concentration ie in 20 µg/ml. 20 µg/ml at this concentration when newly hatched and other instar of larvae were treated and also adults 100 percent mortality is seen. Hence flubendiamide is capable of killing adults as well as larvae within 72hrs of application at very low concentration.

Dead adults emerging from pupae - effect of lufenuron

Larvae pupae intermediate – effect of lufenuron

Shrunken larvae – effect of flubendiamide

CONCLUTION: Toxicity of flubendiamide is high as compared to lufenuron. Flubendiamide shows positive results on every instar of larva at very low concentration and also on adults while lufenuron shows positive effect on larvae at comparatively high concentration with flubendiamide it does not show positive results on adults as compared to flubendiamide. Lufenuron shows is positive results after 48 hours of application while flubendiamide shows positive results within 24 hours of application concluded.

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