EFFECT OF HERBAL PLANT (*Tagetes erecta)* LEAF OIL ON DEVELOPMENTAL STAGES OF WILD *Drosophila melanogaster*

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ABSTRACT

Studies were Conducted to know the efficacy of herbal Plant Tagetes erecta leaf oil against the Wild Drosophila melanogaster by Investigation method. The marigold leaf oil that fulfill the shown insecticidal property against the selected insect Wild Drosophila melanogaster. It is suitable model insect for these studies in laboratory because it is easy to culture with short life cycle of 10 to 14 days and global distribution. The Lc_{50} *of Leaf oil have been calculated as 105.5µl/100ml food. The leaf oil has been considered to observe effect on developmental stages of Wild Drosophila melanogaster. The result clearly indicate negative role of leaf oil of Tagetes erecta on fecundity, larva, pupa and adult count of wild Drosophila Melanogaster. A significance (P<0.01) reduction has been observed all developmental stages, so the Leaf oil of Tagetes erecta is quite effective and has potential in insect control operation. The leaf oil can be a fulfill replacement modern synthetic pesticides.*

Key words *:- Disruption of developmental stages, Tagetes erecta leaf oil, wild Drosophila melanogaster.*

I. INTRODUCTION

Indian economics mainly depends on agriculture and for which fertilizers and high yielding seeds have continuously been used. Inspite of it, insect pests cause considerable damage to agricultural crops. Thus it becomes necessary to prevent this damage by employing various control measures. Pest control has been possible through a number of ways, however, chemical control ranks the first. Although chemical control has provided desired results to some extent in the last few decades yet the outcome in form of environmental problems can not be underestimated. Most of the chemical pesticides are not properly biodegraded and reveal hazardous effects for human beings (Saxena *et al* 2006).

There is now overwhelming evidence that some of these chemicals do pose potential risk to human and other life forms and unwanted effects in the environment. No segment of the population is completely protected against exposure to pesticides and the potentially serious health effects, though a disproportionate burden is

shouldered by the people of developing countries and by high risk group in each country. The world wide death and chronic illness due to pesticide poisoning is about 1 million per year. The government of India has taken steps to ensure the safe use of pesticides. The Insecticide Act, promulgated in 1968 and enforced on 1st August, 1971, envisages to regulate the import, manufacture, sale, transport, distribution and use of insecticides with a view to prevent risks to human beings or animals and for matters connected therewith.

The insect control, in fact, includes all measures that keep a check on feeding, reproduction and dispersal of insect, so as to lead either to their complete eradication or drastic suppression of population. However, no control procedure has brought about 100% reduction in questioned pest population.

Now a days in the chemical control of pests, synthetic organic pesticides dominate the scene. Extensive use of certain synthetic organic insecticides is hazardous to the environment. Also disease vector and pests species have become physiologically resistant to many of these chemical compounds. Currently there are 165 pesticides registered for use in India. There is a sequential rise in the production and consumption of pesticides in India during the last three decades. However, the consumption pattern of these chemicals in India differs with rest of the world. The domestic demand in India accounts for about 76% of the total pesticides used in the country against 44% globally (ICMR, 2001).

There is an urgent need to explore and utilize naturally occurring products for combating insect pests. Before using a new product in field, it is necessary to study its properties, effects on non-target as well as target species, environmental safety and other consideration with many aspects. Though the synthetic organic pesticides are in vogue yet the plant origin pesticides and their analogs have an upper hand. Keeping in mind these views and need of safe and effective pesticides turned our efforts to herbal plant products.

The Plant Word Comprises of a rich storehouse of biochemicals or secondary metabolites. These secondary metabolites are the sources of fine chemicals, Such as drugs, insecticides, dyes, flavones and fragrances. The traditional knowledge on medicinal plants is becoming more popular all over the world providing remedy for all diseases that may afflict human being. The world Health Organization estimate that 80% of the people in developing Countries of the world rely on traditional medicine for their primary health care needs, and 85% of traditional medicine involves the use of plant extracts (Retham and Martin 2006). The plant derived natural products are extensively used as biologically active compounds, particularly in the area of infectious diseases, which represents a serious problem to health, being one of the main cause of mortality worldwide. The environmental problems associated with the large scale use of conventional insecticides have prompted the use of plant oils as an important alternative strategy for the control of mosquito larvae. Since they are a rich source of bioactive compounds, Which are biodegradable to non-toxic producted and potentially suitable for use in integrated insect control programs (Srivastava, *et al.* 2006)

The *Tagetes erecta* (Family, Compositae) has shown both larvicidal as well as adulticidal activity against mosquito (Perich *et al*. 1994) The herbal plant, *Tagetes erecta* which is used for controlling the insect pests having active components, which have been isolated from different parts of the plant.

The sub lethal effects of Leaf oil on wild *Drosophila Melanogaster* has been assessed in various cross combinations to assess the extent of effectiveness of the oil in terms of fecundity, hatchability, pupation and adult emergence. Wild fly has been used as a model insect in the present investigation because it possesses an abundance of the genetic variability, is a highly prolific and is a convenient organism in biological research particularly in genetic and toxicological studies. It is easy to handle and well understood. It is small insect with a short life cycle of 10 to 14 days at 25◦c to 50% relative humidity. The present paper includes toxic response of *Drosophila Melanogaster* to leaf oil.

II. MATERIALS AND METHODS

The Wild *Drosophila Melanogaster* was cultured and maintained in Toxicology Laboratory, Department of Zoology, Dr.B.R.Ambedkar University (Agra) and reared in glass culture vials of 100ml capacity. Wild flies were fed with mixture of distilled water, Agar-agar, corn flour, sugar, yeast, nepazine, propionic acid and 70% alcohol respectively New synthesized *Tagetes erecta* Leaf oil was extracted by hydrodistlation process.

The culture of wild fly and all experiments were conducted inside the B.O.D. incubator at a temp. of 25◦c and 50% relative humidity. Adults flies were used for experimentation and were fully acclimatized to the laboratory conditions. For bioassay procedure, wild flies were divided into five sets, each set consisting of randomly selected 10 individual. The *Tagetes erecta* Leaf oil was prepared in acetone and serially diluted up to five cons. Levels i.e.1000, 500, 250, 125, and 62.50μl per 100ml of food respectively. Some amount of acetone was given to control set. The flies were anaesthetized mildly with anesthetic ether before putting inside the bottle such that these could be easily counted daily. The flies were released in bottles containing the treated medium. Each of the bottles were then covered with sterilized cotton plug. A control set of 10 individuals was also released in bottles similarly. The mortality of flies were recorded for each sets after 48 hours. Moribund insect were considered as dead the mortality data thus obtained was subjected to probit analysis (Finney 1971) to LC_{50} calculated values.

For the response of *Tagetes erecta* leaf oil as growth and developmental inhibitor experiment flies. The prepared of *Drosophila Melanogaster* was divide into four groups. Each Group consisting of two culture bottles were marked as :- (i) TM X UTF (ii) TM X TF (iii) UTM X TF (iv) UTM X UTF out of these treated bottles were given the $1/10^{th}$ concentration of calculated Lc_{50} (105.5µl per 100ml of food), While untreated bottles received same amount diluents (acetone). The control set included UTM X UTF, which were run separately for each of treated sets. The flies were etherized for the separation of males and females flies. Either of saxes were sorted by hand lens and were gently transferred into the bottle after sorting the flies as 10 males and 10 females. Flies were kept separately in Culture bottles that were marked previously and were kept under observation for 3 consecutive days. After 3 days the flies were crossed as :- (a) TF X UTM were placed in place food (b) TM X TF were placed in mixed with *Tagetes* leaf oil food (c) TM X UTF were placed in plane food (d) UTF X UTM were placed in plane food.

All sets were kept inside B.O.D incubator and were to fertilize for 3 days. After three days while the flies were discard. Each set was run in triplicate. The egg hatched into larvae, the third instar larvae came out from the food and stopped feeding, started crawling on the wall of bottles an counted. The larvae were transformed in the pupae. Pupation was considered to begain when the anterior spiracles were everted and the short brood shape of the pupa was formed.

All data obtained were subjected to statistical analysis. The statistical calculations were based on biological statistical formula given by fisher and Yates (1963) Anova Followed by D.M.R.T. was used to determine significance (Bliss 1970, Gad 1999). Anova of toxicity of *Tagetes erecta* leaf oil on wild *Drosophila Melanogaster.*

III. RESULT AND DISCUSION

The Lc_{50} Value of *Tagetes* leaf oil was 105.5 ml 100ml of food against *Drosophila Melanogaster*. Treatment of *Drosophila Melanogaster* with leaf oil *Tagetas erecta* produce Concentration depending toxicity (Table 1).

Table-1

Toxicity evaluation of Leaf oil of *Tagetes erecta* against wild *Drosophila Melanogaster*

Source of oil	Regression equation	Variance	Lc_{50} (In µl/100ml food)	Fiducial limits
Leaf	Y=5.41+2.15(X-2.23)	105.50µl	0.012	M_1=(+)2.043 M_2=(-)1.997

Result of ANOVA of toxicity of leaf oil of *Tagetes erecta* on the wild *Drosophila Melanogaster.* In Various cross combination observation in fecundity, hatchability, pupation and adult emergence. Wild flies were recorded after leaf oil intoxication at sub lethal effect.

In Various Cross combinations, fecundity, hatchability, pupation and adult emergence was decreased after *Tagetes erecta* intoxication. However, more reduction was observed in those cross combination where both sexes were treated as compared to control set.

The decrease in number of eggs is due to the adverse effects of leaf extract on the gonadotropic cycle (Dimetry *et al,*1995). The abnormalities in eggs have also been observed in the treated set as compared to control set. The reduction in number of eggs may also be due to the impaired vitellogenesis and oviposition (sexena and Srivastava, 2002). Volatiles have been seen to impair sensory activities related to oviposition *Tagetes erecta* extrect is also volatiles which can impure the sensory activities related to oviposition (Dhar *et al.* 1996). In the present study, higher mortalities have also be observed during hatchability of larvae (Kalyanasundaram and Das,1985).

In various cross combination, hatchability was reduced after treatment with *Tagetes* Leaf oil, whereas more reduction in number of larvae were observed in those cross combinations, where both sexes have been treated in the present study, higher mortalities have also been observed during hatchability of larvae.

A reduction in number of larvae may possibly be due to the mortality of larvae at the time of moulting because some larval abnormalities have been observed in treated sets which suggest that *Tagetes* leaf oil can like a chitin synthesis inhibitor or like insect growth regulator. The decrease in number of larvae may possibly be due to swelling at the anal papillae in larval bodies, suggesting possible interruption of osmotic and ionic regulation (Clement, 1992). Some larvae that moulted successfully died owing to failure of sclerotization. The decreased number of larvae is also an outcome of the embryonic mortality just before parturition and failure of ecdysis appear to be a major cause of inappropriate adult reproduction (Tang *et al.* 2001)

Reduction in number of larvae may possibly be due to the Leaf oil concentration, easy penetration through delicate covering like chorion and vetelline membranes so the eggs are not converted into the larvae (Dwivedi and Garg 2003).

In Various cross combinations, pupation has been observed to be decreased in all treated sets. However more reduction in number of pupae was observed in those cross combination where both saxes were treated as compared to control set and gain reveled who support by Saxena *et al.* (1993). The decrease in number of pupae may possibly be due to the death during moulting of larvae into pupae. In the present study some deformed pupae have also been observed which in turn resulted in the reduction of pupal count. The present findings are in affirmation to (Saxena and Srivastava 2002).

Further, the reduction of pupae may possibly be due to the failure of sclerotization after moulting. This Suggests that extract interferes with the hormonal control of moulting and possibly due to eclosion with increased concentrations. The observed pupal deformities may be the cause during moulting of larvae into pupae (Sagar *et al*. 1998).

The Reduction in adult emergence following all treatment crosses has been an outcome of adverce effects of experimental oil on apolytic process (Saxena and Srivastava,2002). Further, the decrease in number of adult emergence is due to the formation of larval pupal intermediates with blackening in the integument, incomplete adult emergence in wild *Drosophila Melanogaster* (Sagar *et al*. 1998).

The reduction in adult emergence following all treatment crosses may be considered an outcome of adverse effect of experimental extract on apolytic process and gain support by the observations of Saxena and Srivastava (2002). The reduced adult emergence is in conformity with Saxena *et al*. (1992) who reported decreased adult emergence possibly due to effect on gonadotropic cycle.

Further, reduction in number of adult emergence may possibly be due to the toxic substance present in the extract which has an adverse effect on apolytic process, in which the epidermal tissue is retracted from the old cuticle and which is apparent in the present investigation after treatment with extract. Preset findings are in

agreement with Dwivedi and Garg (2003) who used the flower extract of *Lantana camara* on *Corcyra cephalonica*.

Table-2

ANOVA Following by duncan's multiple range test for Comparing developmental Stages in various cross combination of *Drosophila Melanogaster* Following Treatment of *Tagetes* Leaf oil.

S.N	Sets	No Of Egg laying	No Of Larvae Hatched	No Of Pupa Formed	Adult Emergence
1	TMXUTF	117.33*	100.33*	88.33*	80.66*
2	TFXUTM	101.33**	85.66**	7.33**	68.33**
3	TFXTM	92.33***	72.00****	63***	55***
4	UTFXUTM	137	127	121	114.33

TM=Treted Male, TF= Treated Female, UTM= Untreated Male, UTF=Untreated Female

*=Non-Significant (P>0.05), **=Significant (P>0.05), ***= Highly Significant (P<0.01).

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