International Journal of Advanced Technology in Engineering and Science
Vol. No.4, Issue No. 10, October 2016
www.ijates.com
ijates
ISSN 2348 - 7550

CHEMICAL COMPOSITION AND BIOCIDAL ACTIVITY OF ESSENTIAL OIL FROM TAGETES ERECTA AGAINST DROSOPHILA MELANOGASTER

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ABSTRACT

The Essential oil from leaf of Tagetes erecta were obtained by hydrodistilation process and application of Targets erecta oil to silica gel column chromatgraphy Produce three fractions with haxene. The fractions were applied on Drosophila melanogaster; it is a suitable model insect for these studies in laboratory at during year 2007. Drosophila is easy to culture with short life cycle 10days and global distribution. Drosophila melanogaster has been used to observe the sub lethal effect of Ist, IInd and IIIrd fractions of leaf oil of Tagetes erecta. The Lc_{50} of three fractions have been calculated as 63µl, 43µl and 69µl/100 ml of food. In which IInd fraction more effective as compared to Ist and IIIrd fractions. The IInd fraction has been used present investigation. The fractions were tested with organic solvent and test reagents. The fractions reveled Alkaloid, Flavonoid and Tennin respectively. Adult flies were fed 2days and prescribed food treated with sub lethal concentration is 43µl(1/10^{th} of calculated Lc_{50}) of IInd fraction and allowed to fertilized for 3days and complete the pre-developmental stages. The IInd fraction(Flavonoid) produce toxicity against Drosophila melanogaster.

Keywords: Alkaloid, Flavonid, Tennin and Drosophila Melanogaster.

I. INTRODUCTION

Mostly, Insects are harmful for Agriculture, crops and Storage pest account for nearly 18% less in the productivity of crops, with at current price amount Rs.600 billion annually. The Share by weeds alone is nearly 40% in India, the pests are primarily controlled by chemical and manual means. However, in view of labour becoming unavailable and expensive, the use of chemicals is being increasingly recognized and effective and economical alternative.

Importance of pest control cannot be overemphasized. It has been a constant Endeavour of entomologists to control pests. If not to eradicate them; insects cause losses in uncounted ways. The infestations reduce the yield of crops, lower the quality of food grains and simultaneously increase the cost of production. Therefore, there is a need to apply control measures to prevent the damage caused by insect pests to various crops in the field. Various control methods chemical, mechanical, cultural (Such as crop rotation) sterile male release, biological use of microwave etc. have been employed by them. However, all these methods have their limitations.

The Chemicals insecticides are generally applied to the crop in the from of liquid spray dusts. But only one percent of the applied insecticides hits the targets insect/pests while the remaining part drifts in the environment or gets accumulated in to the food chain. Thus, the insecticides and pesticides pose a potential health hazard, not only to live stock and wild life but to all including human being. Besides, the adverse effects on the Environment and the Consumers, chemicals control alone is not enough. Alternate methods to check the losees by pest are, therefore, badly needed.

Biological control of insect pest, Using natural products is one such alternate, which is economically very cheap and also environmentally friendly. It has been noted that various plants are avoided by insect pests. This is because these plants contain some chemical substance such as alkaloids, flavonoid and tennin. If the extract from such plants is used as insecticide, this would be the safest way of the pest control, because such abstracts are very cost effective non phytotoxic and leave residual hazard for insect.

The herbal plant product, which in the present investigation has been obtained from *Tagetes erecta* possessing some active components for controlling insect pests. The sub lethal concentrations of active components of leaf oil have been found to be effective on wild *Drosophilla melanogaster*. The selection of *Drosophilla melanogaster* is based on this fact, it possesses an abundance of the genetic variability, is highly prolific, is a convenient and inexpensive organism. It is a special insect to be studied in biological, genetical and toxicological laboratories throughout the world due to its cycle of 10-14 days at 25^0C, easy to rear handle and has global distribution.

II. MATERIALS AND METHODS

Hydrodistilation:-The plant leaves were collected from botanical garden, cut into small pieces and washed with distilled water and dried under shade. The oil was obtained from the leaf powder with the help of soxhlet apparatus.

Chromatographic procedures: - The leaf oil was subjected by column chromatography carried out and silica gel (mesh size 60-120) using hexane as solvent system. Column chromatography produced three fractions. All fractions were then tested for detecting the presence of chemical groups. Mayer's reagents and alcoholic oil were used for Alkaloids, Flavonoids through ammonium salt, lead acetate and hydrochloric acid and Tannins by lead acetate, aqueous bromine solution and acetonic acid. The Ist, IInd and IIIrd tractions revealed the presence of Alkalods, Flavonodis and Tannins respectively.

Bioassay:- The most active fractions (Flavonoids) were evaluated against egg and larva *Drosophilla melanogaster*. It was cultured and mainted in toxicology laboratory, Dept. of Zoology Dr. B. R. Ambedkar university, Agra (India) and reared in glass culture bottles of 100 ml of capacity and maintained under the laboratory conditions. Wild flies were fed with mixture of distilled water, Agar-agar, corn flour sugar, yeast, nepazine, propeonic acid and 70% alcohol. The Lc_{50} of chromatographic fractions were calculated by probit analysis (Finney, 1971).

There were four groups (i) TM X UTF (ii) TM X TF (iii) UTM X TF (iv) UTM X UTF with three sets for each cross. All sets kept inside B.O.D. incubator and were allowed to fertilize for 3 days, after consecutive days flies were discarded eggs and Larva, were counted regularly.

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, Gad1999)

III. RESULT AND DISCUSION

The Lc_{50} Value of active fractions of *Tagetes erecta* leaf oil was 63µl, 43µl, 69µl /100ml of food against *Drosophila Mealangaster*. The active fraction (II^{nd}) has been observed to be more toxic as compared Ist and IIIrd fractions of leaf oil on the basis of Lc50 value. Treatment of *Drosophila Mealangaster* with the IInd fraction of leaf oil of *Tageta erecta* produces Concentration depending toxicity (Table 1)

Table-1

Toxicity evaluation fractions of leaf oil of Tagetes erecta on Drosophila Mealangaster

Source of *Tagetes erecta* leaf oil	Regression equation	Variance	Lc_{50} (In µl/100ml food)	Fiducial limits
First fraction	Y=5.38+2.16 (X-2.02)	0.015	69.18µl	M_1=(+)1.869 M_2=(-)1.546
Second fraction	Y=5.42+1.78 (X-1.87)	0.018	43.65µl	M_1=(+)1.669 M_2=(-)1.611
Third fraction	Y=5.28+2.18(X-1.96)	0.013	63.65µl	M_1=(+)1.869 M_2=(-)1.771

Result of ANOVA of toxicity of second fraction of leaf oil of *Tagetes erecta* on the wild *Drosophila Mealangaster*. In Various cross combination observation in fecundity, hatchability and pupation. Wild flies were recorded after Second fraction of leaf oil intoxication at sublethal effect.

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

In present study, higher mortalities have also been observed during fecundity of eggs. 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 various cross combination, hatchability was reduced after treatment with second fraction of *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 second fraction of *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 second fraction of leaf oil 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)

Table-2

ANOVA Following by duncan's Multiple rangetest for Comparing developmental Stages in various cross combination of *Drosophila Mealangaster* Following Treatment of second fraction of *Tagetes* Leaf oil

S.N	Sets	No Of Egg laying	No Of Larvae Hatched	No Of Pupa Formed
1	TMXUTF	107.33*	94.33*	75.33*
2	TFXUTM	91.33**	71.66**	56.33**
3	TFXTM	82.33***	64.00****	47***
4	UTFXUTM	119	95	84

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

*= Significant (P>0.05), **= Highly Significant (P>0.05), ***= Very H ighly Significant (P<0.01).

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