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Feeding deterrence and toxicity of N- (phenyl) -2 hydroxy salicylimide and N-(p-bromophenyle)-2hydroxy salicylimide against pulse beetle, *Callosobruchus chinensis* L. (Bruchidae) on chickpea under laboratory

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Abstract
The present manuscript covers the laboratory stomach toxicity of new synthesized amides of $C_{13}H_{11}NO_2$, $C_{13}H_{10}NO_2Cl$, $C_{13}H_{10}NO_2Br$, $C_{14}H_{13}NO_2$ and $C_{14}H_{13}NO_3$ on seeds of chickpea, *Cicer arietinum* L.ver. K 850 were treated with different concentrations (0.06%, 0.12%, 0.25%, 0.50%, and 1.00%) levels against the attack of chickpea bruchid, *Callosobruchus chinensis* Linn. (Coleoptera: Bruchidae). These compounds are found effective in in controlling the insect infestation in chickpea under storage condition.In the present paper we describe the insecticidal activity of these amides against early emerged adults of *C. chinensis* L. of both male and female, respectively. Successful adoption of new synthesized amides of salicylic acid in the protection of food commodities promises an eco-friendly option compatible with international biosafety regulations.The newly synthesized amides compound exhibited lethal concentration and LC_{50} values in following respective descending order as: N-(phenyl) -2 hydroxy salicylimide (0.60) > N- (p- bromophenyle) -2 hydroxy salicylimide (0.68) > N- (p-tolyl) -2-hydroxy salicylimide (1.10) > N- (P-methoxy Phenyl-2 hydroxy salicylimide (1.20) > N-(p-chlorophenyle) -2 hydroxy salicylimide (2.97), respectively. It was found that all the compounds show moderate efficacy against the insect.

Keywords: insecticidal bioefficacy, *Callosobruchuschinensis*, amides of sulphosalicylic acid

1. Introduction

The chickpea (*Cicer arietinum* L.) is a major grain legume cultivated for its edible seeds in the Mediterranean, oriental and Australiean zoogeographical region. The world's total production of chickpeas hovers around 8.5 million metric tons annually and is grown over 10 million hectares of land approximately. Our country is the largest producer of this pulse contributing to around 70% of the world's total production. Chickpea,*Cicerarietinum* (Leguminosae) is fifth most important legume crop in the world it ranks third among the 8.5 million metric tons world's pulse crops after dry bean and dry pea, *C. arietinum* is grown in India over 10 million hectares of land approximately [1]. The Desi type chickpea contribute to around 80% and the Kabuli type around 20% of the total production. As per FAO (Food and Agriculture Organisation (FAO) report the pule production is about 12 million metric tons is 2011 of total world production [2].

A significant part of the world population relies on legumes as staple food particularly in combination with cereals. Chickpea (*Cicer arietinum* L.) are one of the oldest and most widely consumed legumes often advocated in human diet because of their beneficial nutritional effects and a low cost protein source [3]. The protein content of legume grains is relatively high but its protein quality is low [4]. Chickpea is a multipurpose grain legume, notably as a major and cheap source of protein compare to animal protein. Pulses contain 20-30% of protein and some essential amino acids. The pulse protein is three times higher than that found in cereals [5]. Among the insect pests attacking stored products, pulse beetle, *C. chinensis* is a serious one [6, 7].

Chickpea has been reported to cause serious damage to pulses in India and many countries of the world [8].As it is evident that *Callosobruchus* spp. cause heavy losses every year and affect the economy of the country, suitable control measures should be taken against them. The pulse seeds suffer a great damage during storage due to insect attack. Many entomologist reported that pulse beetle, *C. chinensis*is a destructive pest of chickpea under storage conditions [9, 10, 11, 12]. In India like developing countries the greatest losses during storage to cereals and grain legumes are caused by insect pests. Insect pest control in durable stored agricultural produce at farm level is increasingly relying on the use of certain insecticides such as fenthion, lindane, endosulfan and DDT by farmers who lack technical knowledge in the safe handling and use of such products. The misuse of synthetic pesticides has led to accidental poisoning, the development of insect resistance and other adverse environmental and health hazards [13].

In recent year literature survey revealed that amines in general have been known to be biologically active [14] and the effect of presence of various constituents in the some new synthesized amides viz; $C_{13}H_{11}NO_2$, $C_{13}H_{10}NO_2Cl$, $C_{13}H_{10}NO_2Br$, $C_{14}H_{13}NO_2$ and $C_{14}H_{13}NO_3$ increases insecticidal activity, has been investigated [15, 16]. Insects are found in almost all types of environment. The compounds containing amide moiety has also attracted attention due to their important role as insecticides and pesticides to save our Indian crops [17]. In view of the, important behavior of amines and amino acid, the present communication deals with the synthesis, characterization and insecticidal activity of some new amide of salicylic acid against early emerged adults of *C. chinensis* Linn [18].

2. Materials and Method

Experiments were conducted to test the toxic effect of four amides of salicylic acid in the Bio-pesticide and herbal extract laboratory, Department of Zoology, Entomology, D.B.S.College, Kanpur Affiliated to C.S.J.M University, Kanpur, India. In the present investigation four amides of salicylic acid were prepared. Equimolar quantities of aromatic amine and salicylic acid are taken in a hard glass tube and heated over oil bath for 6-7 hours. After completion of reaction, product poured in cold water, lumps are formed, then crushed and wash with cold water. Finally, re-crystallized with ethanol -water (v/v) solution. Brownish needle shaped crystal are formed. Which are identified as N (phenyl) -2 hydroxySalicylimide, N- (p-talgyl) -2-hydroxy Salicylimide, N-(p- chlorophenyle) -2 hydroxysalicylimide and N-(P-methoxy Phenyl-2 hydroxySalicylimidexx, respectively.

2.1 Rearing of the test insect

Adults of *Callosobruchus chinensis* were drawn from laboratory mass cultures reared in glass jars at temperature 25±1°C without controlling conditions. Mass culture of *C. chinensis* was maintained using the procedure described by Strong *et al.* (1968). Adults emerged from this culture were used for the bioassay within 48 hrs to 72 hrs of emergence and fed on chickpea (channa).

2.2 Apparatus used for experiment

Small plastic jars (capacity 50 ml) were used for the experiment; there was one set of two jars joined by clear plastic pipe of 1 cm diameter at an angle of 180 degree for each replication. One jar of each set was provided with 10 g of grains given the name 'T' while the other jar was kept empty and given the name 'C'. In jar 'T', the grains treated with extracts were placed, while the jar B remained empty. The jars used for experiment were disinfected with alcohol.

2.3 Experimental Protocol Test

The stomach toxicity of these compounds was carried out by leaf dip method [13]. In this method we used early emerged adults of *C. chinensis* Ten adults beetle were used for three treatments and each treatment with three replications were maintained for each concentration. The given compounds were dissolved in methanol and different concentrations were prepared viz. 0.06%, 0.12%, 0.25%, 0.50%, and 1.00%. The leaf disc were prepared out of chickpea seeds and dipped in various concentrations of the test compounds for thirty seconds. Now air dried the chickpea seeds discs to evaporate the excess acetone. The leaf discs dipped only in acetone were served as control. The mortality data was recorded after 24 hrs. and the treatment mortality was corrected with control mortality. These mortality data was used for calculation of LC_{50} values [20].

3. Results

The amide was generally prepared by using following method:-

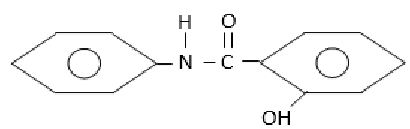
Aromatic amine + salicylic acid (in water bath reflux) --- Amides

The newly prepared compounds were crystallized after vacuum distillation. The compounds have sharp melting point. The further characterization of these entire compounds was done by elemental analysis, I.R and NMR spectral data followed by insecticidal activity as N (phenyl) -2 hydroxy salicylimide, N-(p- chlorophenyle) -2 hydroxysalicylimide, N-(p-talgyl) -2-hydroxy salicylimide, and N-(P-methoxy Phenyl-2 hydroxySalicylimide,N-(p- bromophenyle) -2 hydroxysalicylimide, respectively.

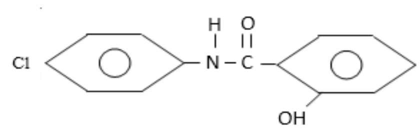
3.1 Structural and Molecular Formula

The structural formula of the synthesized new synthesized amides compounds are given below:

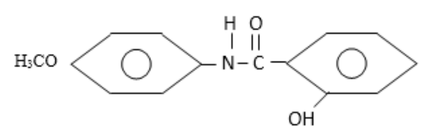
1. $C_{13}H_{11}NO_2$ [N- (phenyl) -2 hydroxy Salicylimide]



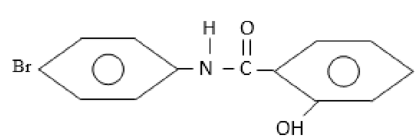
2. $C_{13}H_{10}NO_2Cl$ [N- (p- chlorophenyle) -2 hydroxysalicylimide**]**



3. $C_{14}H_{13}NO_3$ [N- (p-methoxy Phenyl-2 hydroxy salicylimide]



4. $C_{13}H_{10}NO_2Br$ [N-(p- bromophenyle) -2 hydroxysalicylimide



5. $C_{14}H_{13}NO_2$ [N- (p-tolyl) -2-hydroxy salicylimide]

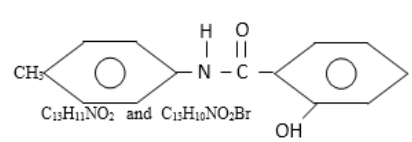


Table 1: Stomach toxicity of early emerged adults of *C. chinensis* due to amides of salicylic acid at 24 hrs

Organic Compounds	Molecular formula	Chi-square (X^2)	Slope (Y)	LC_{50}	Fiducial limits	Relative LC_{50}
N (phenyl) -2 hydroxySalicylimide	$C_{13}H_{11}NO_2$	2.97	Y=1.57 + 0.16	0.60	M_1=0.49 M_2= 0.77	4.95
N-(p- chlorophenyle) -2 hydroxysalicylimide	$C_{13}H_{10}NO_2Cl$	0.68	Y=1.01+ 0.17	2.97	M_1=1.61 M_2= 9.55	1.00
N- (p-tolyl) -2-hydroxy Salicylimide	$C_{14}H_{13}NO_2$	0.65	Y=1.45+ 0.17	1.10	M_1=1.82 M_2= 1.67	2.70
N- (P-methoxy Phenyl-2 hydroxySalicylimide	$C_{14}H_{13}NO_3$	0.80	Y=1.28 + 0.16	1.20	M_1=0.86 M_2= 1.99	2.47
N-(p- bromophenyle) -2 hydroxysalicylimide	$C_{13}H_{10}NO_2Br$	3.39	Y=1.49 + 0.16	0.68	M_1=0.54 M_2= 0.90	4.36

In case of X2 was found non significant heterogeneous at P=0.05, Y=Probit Repellency, X=Log Concentration X 102, D.F.=Degree of Freedom, E.C.50= Concentration Calculated at given 50% Repellency and H*=3

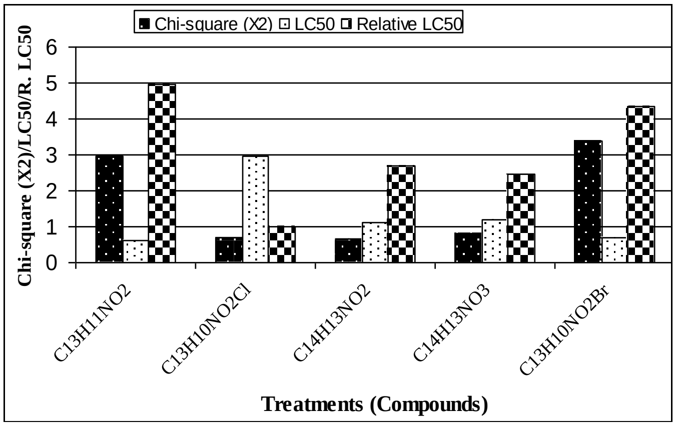


Fig 1: Stomach toxicity of early emerged adults of *C. chinensis* due to amides of salicylic a acid at 24 her

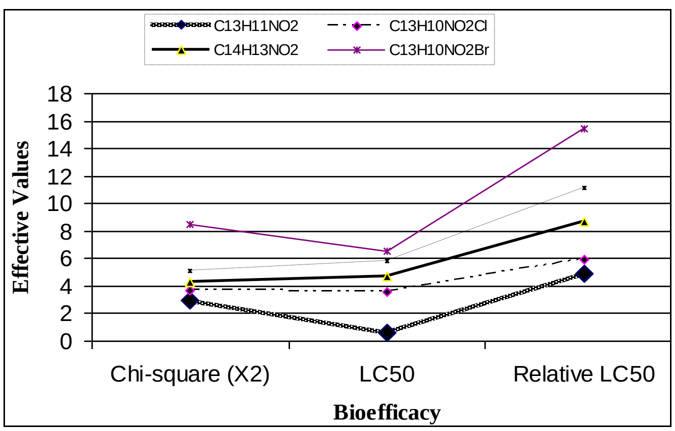


Fig 2: Feeding toxicity of early emerged adults of *C. chinensis* due to amides of salicylic a acid at 24 her

3.2 Insecticidal Effect

The data depicted from table 1 and figure 1 and 2 that among all the newly synthesized amides compound the N- (phenyl) -2 hydroxy salicylimide (0.60) exhibited maximum mean mortality percentage with minimum lethal concentration (LC_{50}) followed by N- (p- bromophenyle) -2 hydroxy salicylimide (0.68) against early emerged adults of *C. chinensis* L.. The lethal toxicity was seen in N- (N- (p-tolyl) -2-hydroxy salicylimide (1.10) and N- (P-methoxy Phenyl-2 hydroxy salicylimide (1.20) to the test beetles, where as N-(p-chlorophenyle) -2 hydroxy salicylimide (2.97) was found least toxic and taken as unit. Over all, selected newly synthesized compounds show moderate efficacy against the *C. chinensis* L.

4. Discussion

For the control of several noxious insect pest many worker used certain amides and other organic safe and natural chemicals as insecticides and reported their significant results [19,20,21,22,23]. Hence, the demand for amides of salicylic acid is enhancing as they are usually recognized to be safer than synthetic insecticides [24,25,26,27,]. In the support of above findings the works of various entomologist and biochemist gave their views that these organic chemicals might be use for the control of insect infestation on crop and under storage condition.[28,29].The amides of salicylic acid can be used as alternative approach to *C. chinensis* control on chickpea. [30,31,32] Many scientific studies have proven that amides of salicylic acid derived products can be used as an alternate approach to control insect pest population [33]. The methanol and water formuation of N (phenyl) -2 hydroxy salicylimide showed highest repellency against early emerged adults of *C. chinensis*. Similarly N-(p- bromophenyle) -2 hydroxy salicylimide showed potential repellency againt early emerged adults of *C. chinensis.* The remaining amides of salicylic acid such as N- (p-tolyl) -2-hydroxy salicylimide, N- (P-methoxy Phenyl-2 hydroxy salicylimide and Hydrocotylejavanica, where as N-(p- chlorophenyle) -2 hydroxy salicylimide taken as unit and showed least effective among all the selected products of amides.

5. Conclusion

Conclusively, the present investigation revealed that there appears prospects in selected amides of salicylic acid might encourage the search for novel, natural organic insecticides offering an alternative to hazourdous toxic synthetic insecticides. The amides of salicylic acid like $C_{13}H_{11}NO_2$ and $C_{13}H_{10}NO_2Br$ have the potential to be used as an ideal approach for the management of pulse beetle, *Callosobruchus chinensis* under storage conditions.

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