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Laboratory bioassay of N- (P-methoxy Phenyl-2 hydroxy salicylimide and N- (p- chlorophenyle) -2 hydroxysalicylimide as repellent against pulse beetle, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae)

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Abstract
In the present investigation experiments were conducted in the Biopesticide and Toxicological plant product research laboratory, Department of Zoology, D.B.S. College, Kanpur, U.P., India. The pago-repellent effects of some new synthesized amides of salicylic acid 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 $C_{14}H_{13}NO_3$ were tested on seeds of chickpea, *Cicer arietinum* L.ver. K 805 against early emerged adults of *Callosobruchus chinensis* Linn. (Coleoptera: Bruchidae) The different concentration (0.06%, 0.12%, 0.25%, 0.50% and 1.00%) levels of amides of salicylic acid against the attack of chickpea bruchid *C. chinensis* Linn. on chickpea seeds. Based on EC_{50} values among all compounds, N- (P-methoxy Phenyl-2 hydroxy salicylimide (0.37) was found most repulsive in controlling the insect infestation in chickpea under storage condition while N-(p-bromophenyle) -2 hydroxy salicylimide (1.34) the least and taken as unit. Remaining amides were recorded based on EC_{50} as potent repulsive effect in respective descending order as:- N (phenyl) -2 hydroxy Salicylimide (0.58).> N- (p-tolyl) -2-hydroxy Salicylimide (0.71) > N- (p- chlorophenyle) -2 hydroxysalicylimide (2.97), respectively. The results indicate that these compounds have the potential repellent biopotential effect to protect chickpea seeds from pulse beetle damage compared to when the untreated seeds. They should, therefore, be included in pest management strategies for pulse beetle in grains stored on-farm in rural tropical and subtropical oriental regions.

Keywords: *Callosobruchus chinensis* amides of salicylic acid, $C_{14}H_{13}NO_3$ and repellent

1. Introduction

The total global production of pulses as per Food and Agriculture Organisation (FAO) report is approximately 12 million metric tons [1]. India is the largest producer of pulses contributing to around 70% of the world's total production. Chickpea, Chickpea, Cicer *arietinum* (Leguminosae), is an important edible legume crop in many parts of the world especially in tropical and subtropical regions *Cicer arietinum* (Leguminosae) is fifth most important legume crop in the world it ranks third in world's pulse crops production. It is is grown in India approximately over 10 million hectares of land [2].

It is used as human food due to its high protein content and also as livestock feed to make silage and hay. Chickpea is a major and cheap source of protein compare to animal protein. Pulses contain 20-30% of protein and some essential amino acids, enrich the soil through Symbolic nitrogen fixating from atmosphere. The pulse protein is three times higher than that found in cereals [3]. Many entomologist reported that pulse beetle, *C. chinensis* is a destructive pest of chickpea under storage conditions [4]. Chickpea has been reported to cause serious damage to pulses pulse seeds during storage in India and many countries of the world [5]. Pulse production is affected by number of insect pests infestations which lead to economic losses. Insect damage is the major constraint to chickpea grain production in most pulse producing countries [6].

The major insect pests that can cause economic loss are pulse beetles species under storage conditions. The Desi type chickpea contribute to around 80% and the Kabuli type around 20% of the total production. There are many species of pulse beetle infesting under storage condition on chickpea viz; *Callosobruchus chinensis, Callosobruchus maculatus* and *Callosobruchus analis.* Among them pulse beetle, *Callosobruchus chinensis* Linn. (Coleoptera : Bruchidae) is a serious one [7, 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 [9, 10]. In recent year literature survey revealed that Amines in general have been known to be biologically active [11] and the effect of presence of various constituents in the amines increases insecticidal activity, has been investigated [12, 13]. Insects are found in almost all types of environment. The compounds containing amide moiety has also attracted attention due to their important role as repellent and insecticides to save our crops and stored products [14, 15].

In view of the, important behavior of amines and amino acid, an attept has been taken to synthesize some new amide of salicylic acid viz; N- (P-methoxy Phenyl-2 hydroxySalicylimide, N (phenyl) -2 hydroxy Salicylimide, N- (p-tolyl) -2-hydroxy Salicylimide, N- (p- chlorophenyle) -2 hydroxysalicylimide and N- (p- bromophenyle) -2 hydroxy salicylimide as repellent for the control of early emerged adults of *C. huschinensis* under laboratory conditions [17].

2. Materials and Method

The experiments were conducted in biopesticide and plant product laboratory, Department of Zoology, D.B.S. College, Kanpur affiliated to CSJM Uuniversity, Kanpur. The amide was generally prepared by using following method:

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

2.1 Experimental Protocol

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 alcohol-water (v/v) solution. Brownish needle shaped crystal are formed. Which are identified as N (phenyl) -2 hydroxy salicylimide, N- (p-talgyl) -2-hydroxy salicylimide, N-(p-chlorophenyle) -2 hydroxysalicylimide, N-(P-methoxy Phenyl-2 hydroxy salicylimide and and N- (p- bromophenyle) -2 hydroxy salicylimide, respectively.

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 1cm 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 'A' while the other jar was kept empty and given the name **'**B**'**. In jar 'A**'**, the grains treated with 0.06%, 0.12%, 0.25%, 0.50% and 1.00% concentrations of amides of salicylic acid were placed, while the jar B remained empty. The jars used for experiment were disinfected with alcohol.

3. Bioassay Test

The repellent bioefficacy of these compounds of amides of salicylic acid was also carried out by seed dip method using early emerged adults of *C. chinensis* Linn [18]. There are ten early emerged adults were used for each replication and 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 seeds discs were prepared and dipped for thirty seconds in various concentrations of the test compounds. Now air dried the seed discs to evaporate the excess methanol and the leaf seed discs offered for feeding [19]. The insects were allowed to feed for 24 hrs. After 24 hrs number of treated adult beetle repells from treated food and number of beetle remain on the food and repels from food was measured by counting. The differences between number of beetle introduced and repelled from treated food were counted. The repellency over control was calculated and used for calculation of effective concentration (EC_{50}).

3.1 Statistical Analysis

The repellent effect of all the extracts was judged by counting the number of grubs and adults of beetles after 4 hours present on the treated leaf in each treatment and the percentage of repellency were adjudged over control. For each treatment, the data then was subjected to Probit analysis (Finney, 1952) and the results were compared on the basis of respective, EC_{50} values (Godin *et al*.1965) [16, 30, 31].

Table 1: Calculation of log concentration / probit repellency regression graph: Insect Pest: *Callosobruchus chinensis* L. Insecticidal compound: $C_{14}H_{13}NO_3$

Details	*Concentrations*					Control	Total
	0.06%	**0.12%**	**0.25%**	**0.5%**	**1.0%**		
TNIU	15	15	15	15	15	15	-
TNIRF	7.63	4.54	2.98	2.25	2.75	11.50	-
TNROC	33.65	60.52	74.08	80.43	93.47	-	-
Log X 10^2(x)	1.40	1.70	2.00	2.18	2.30	-	-
Emprove probit	4.57	5.26	5.63	5.82	6.50	-	-
Prove probit	4.5	5.2	5.8	6.1	6.4	-	-
Working 'Y'	4.57	5.26	5.63	5.82	6.50	-	-
Weight 'X'	0.5809	0.6274	0.5226	0.4047	0.3019	-	-
Nw	8.71	9.41	7.53	6.07	6.52	-	38.24
Nwx	12.19	15.99	15.06	13.37	10.39	-	67.00
Nwy	39.80	49.49	42.39	35.32	29.38	-	196.38
Nwx^2	17.07	27.19	30.12	28.83	23.81	-	127.02
Nwxy	55.72	84.13	84.78	76.99	67.57	-	369.19
Nwy^2	181.90	260.35	238.67	205.60	190.97	-	1077.49
$\bar{X}$ =1.73 X^2 = 3.48 Y=b x + c V(m)=0.0336 (m_1)= 0.3034 =1.25 $\pm$ 0.14 Fiducial Limit							
$\bar{Y}$ = 5.89 b = 0.61 EC_{50} = 0.37 SE(m) =0.1832 (m_2)=0.4812							

TNIU= Total number of insect used. TNIRF = Total number of insect reached, TNROC= Total number of repellency over control

Table 2: Calculation of log concentration/probit repellency regression radar: Insect Pest: *Callosobruchus chinensis* L. Insecticidal compound: $C_{13}H_{10}NO_2Br$

Details	**Concentrations**					Control	Total
	0.06%	**0.12%**	**0.25%**	**0.5%**	**1.0%**		
TNIU	30	30	30	30	30	30	-
TNIRF	20	15	14	11	08	29	-
TNROC	31.03	48.28	51.71	62.07	72.41	-	-

Log X 10^2(x)	1.40	1.70	2.00	2.18	2.30	-	-
Emprove probit	4.50	4.95	5.04	5.30	5.59	-	-
Prove probit	4.5	4.7	5.0	5.3	5.6	-	-
Working 'Y'	4.50	4.95	5.04	5.30	5.59	-	-
Weight 'X'	2.5809	0.6343	0.6366	0.6160	0.5809	-	-
Nw	17.43	19.03	19.10	18.48	17.43	-	91.47
Nwx	24.40	32.35	38.20	40.29	40.09	-	175.33
Nwy	78.44	94.20	96.26	97.29	97.43	-	463.62
Nwx^2	34.16	55.00	76.40	87.83	92.21	-	345.60
Nwxy	109.80	160.13	192.53	213.54	221.40	-	897.4
Nwy^2	352.93	466.29	485.15	519.08	544.63	-	2368.08

$\bar{X}$=1.9168 X^2= 0.703 Y=b x + c V(m)=0.009886 (m_1)=0.84275 =1.06 ± 0.15 Fiducial Limit
$\bar{Y}$= 5.0756 b = 1.0692 EC_{50}=1.24 SE(m) =1.84604 (m_2)=2.34325

Table 3: Calculation of log concentration/probit repellency regression graph Insect Pest: *Callosobruchus chinensis* L. Insecticidal compound: $C_{13}H_{11}NO_2$

Details	Concentrations					Control	Total
	0.06%	**0.12%**	**0.25%**	**0.5%**	**1.0%**		
TNIU	15	15	15	15	15	15	-
TNIRF	6.37	4.76	3.68	3.66	2.043	12.10	-
TNROC	47.35	60.66	69.58	69.75	83.14	-	-
Log X 10^2(x)	1.40	1.70	2.00	2.18	2.30	-	-
Emprove probit	4.93	5.26	5.51	5.51	5.95	-	-
Prove probit	4.8	5.2	5.6	5.8	5.9	-	-
Working 'Y'	4.93	5.26	5.51	5.48	5.95	-	-
Weight 'X'	0.6274	0.6274	0.5578	0.5026	0.4714	-	-
Nw	9.41	9.41	8.36	7.53	7.07	-	41.78
Nwx	13.17	15.99	16.72	16.41	16.26	-	78.55
Nwy	46.39	49.49	46.06	41.26	42.06	-	225.26
Nwx^2	18.44	27.19	33.44	35.76	37.40	-	152.23
Nwxy	64.94	84.13	92.12	89.94	96.73	-	427.86
Nwy^2	228.70	260.35	253.81	226.12	250.29	-	1219.27

$\bar{X}$=1.83 X^2= 1.34 Y=b x + c V(m)=0.845 (m_1)=0.437812 =1.03 ± 0.14 Fiducial Limit
$\bar{Y}$ = 5.90 b = 0.73 EC_{50}= 0.58 SE(m) =0.9192 (m_2)=0.87218

Table 4: Calculation of log concentration/probit repellency regression radar: Insect Pest: *Callosobruchus chinensis* L. Insecticidal compound: $C_{13}H_{10}NO_2Cl$

Details	Concentrations					Control	Total
	0.06%	**0.12%**	**0.25%**	**0.5%**	**1.0%**		
TNIU	15	15	15	15	15	15	-
TNIRF	5.17	5.00	4.19	2.38	2.33	19.07	-
TNROC	55.43	55.89	63.87	39.48	79.91	-	-
Log X 10^2(x)	1.40	1.70	2.00	2.18	2.30	-	-
Emprove probit	5.13	5.17	5.35	5.82	5.83	-	-
Prove probit	5.1	5.3	5.6	5.8	5.9	-	-
Working 'Y'	5.13	5.17	5.33	5.82	5.83	-	-
Weight 'X'	0.6343	0.6260	0.2578	0.5026	0.4714	-	-
Nw	9.51	9.24	8.36	7.53	7.07	-	41.71
Nwx	13.31	15.70	16.72	16.41	16.26	-	78.40
Nwy	48.78	47.77	44.55	43.82	41.21	-	226.13
Nwx^2	18.63	26.70	33.44	35.76	37.40	-	151.93
Nwxy	68.29	81.20	89.10	95.52	94.78	-	428.89
Nwy^2	250.27	246.97	237.49	255.05	240.30	-	1230.08

$\bar{X}$=1.60 X^2= 0.20 Y=b x + c V(m)=0.042614 (m_1)=0.71038 =0.89 ± 0.14 Fiducial Limit
$\bar{Y}$= 4.87 b = 2.727 EC_{50}= 0.58 SE (m) =0.0659 (m_2)=2.213662

Table 5: Calculation of log concentration/probit repellency regression radar: Insect Pest: *Callosobruchus chinensis* L. Insecticidal compound: $C_{14}H_{13}NO_2$

Details	Concentrations					Control	Total
	0.06%	**0.12%**	**0.25%**	**0.5%**	**1.0%**		
TNIU	30	30	30	30	30	30	-
TNIRF	21	20	14	12	10	30	-

TNROC	30.00	33.33	53.33	60.00	66.67	-	-
Log X 10^2(x)	1.40	1.70	2.00	2.18	2.30	-	-
Emprove probit	4.47	4.56	5.08	5.25	5.42	-	-
Prove probit	4.6	4.8	5.0	5.2	5.4	-	-
Working 'Y'	4.47	4.56	5.08	5.25	5.42	-	-
Weight 'X'	0.5578	0.5809	0.6366	0.6274	0.6005	-	-
Nw	16.73	17.43	19.10	18.82	18.02	-	90.10
Nwx	23.42	29.63	38.20	41.03	41.45	-	173.73
Nwy	74.78	79.48	97.03	98.91	97.67	-	447.87
Nwx^2	32.79	50.37	76.40	89.45	95.34	-	344.35
Nwxy	104.69	135.12	194.06	215.41	224.64	-	873.92
Nwy^2	334.27	362.43	492.91	518.75	529.37	-	2237.73
$\bar{X}$=1.92819	X^2 = 0.895	Y=b x $\pm$ c	V (m)=0.0088352	(m_1) = 0.498738	=0.87 $\pm$ 0.13	Fiducial Limit	
$\bar{Y}$ = 4.96970	b=1.2477	EC_{50} = 0.71	SE (m) =1.95512	(m_2) = 1.251520			

Table 6: Summary of Log concentration/probit repellency regression line and histogram: (Bioefficacy of *C. chinensis* due to amides of salicylic acid at 24 hrs)

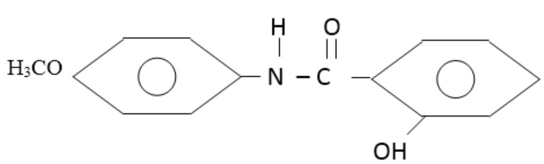
Amides of salicylic acid	Compounds	Chi-square (X^2)	Slope (Y)	LC_{50}	Fiducial limits	Relative LC_{50}
N- (P-methoxy Phenyl-2 hydroxySalicylimide	$C_{14}H_{13}NO_3$	3.48	Y=1.25 $\pm$ 0.14	0.37	M_1=0.30 M_2= 0.48	3.3513
N-(p- bromophenyle) -2 hydroxysalicylimide	$C_{13}H_{10}NO_2Br$	0.70	Y=1.06 $\pm$ 0.15	1.24	M_1=0.84 M_2= 2.34	1.00
N (phenyl) -2 hydroxy Salicylimide	$C_{13}H_{11}NO_2$	034	Y=1.03 $\pm$ 0.14	0.58	M_1=0.43 M_2= 0.87	2.1258
N- (p- chlorophenyle) -2 hydroxysalicylimide	$C_{13}H_{10}NO_2Cl$	0.20	Y=0.89 $\pm$ 0.14	1.08	M_1=0.71 M_2= 2.21	1.1481
N- (p-tolyl) -2-hydroxy Salicylimide	$C_{14}H_{13}NO_2$	0.89	Y=0.87 $\pm$ 0.13	0.71	M_1=0.49 M_2= 1.25	1.7468

In case of X^2 was found non- significant heterogeneous (3) at P=0.05, Y=Probit Mortality, X=Log Concentration X 10^2, D.F. =Degree of Freedom, LC_{50}= Conc. Calculated at given 50% mortality.

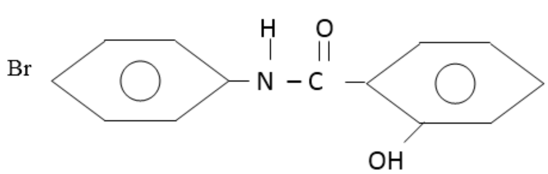
3.2 Structure of newly synthesized amides of salicylic acid

The structure of the synthesized compounds are given as

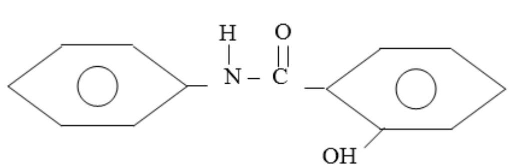
(1) $C_{14}H_{13}NO_3$ [N- (P-methoxy Phenyl-2 hydroxySalicylimide]



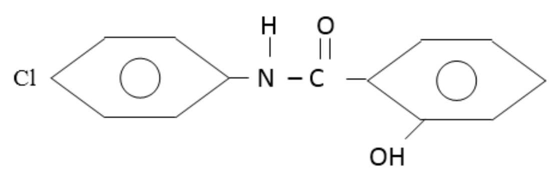
(2) $C_{13}H_{10}NO_2Br$: [N-(p- bromophenyle) -2 hydroxysalicylimide



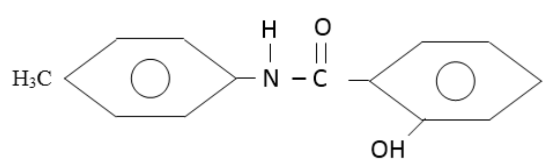
(3) $C_{13}H_{11}NO_2$: [N (phenyl) -2 hydroxy Salicylimide]



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



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



4. Results

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 [20]. These newly synthesized amides such as N (phenyl) -2 hydroxy salicylimide, N- (p-tolyl) -2-hydroxy salicylimide,N- (p-chlorophenyle) -2 hydroxy salicylimide N- (P-methoxy Phenyl-2 hydroxy salicylimide and N- (p-chlorophenyle) -2 hydroxy salicylimide insect-repellent compound exhibited effective concentration based on following respective EC_{50} values in the descending order as : 0.34 >2.01 >2.12 >2.97 and 3.53, respectively It was found that the compounds show moderate repellent effect against early emerged adults of *C. chinensis.* The results depicted from the table-1 and figure 1 that compound N (phenyl) -2 hydroxy salicylimide showed the highest repellency to early emerged adults of *C. chinensis* and N- (p- chlorophenyle) -2 hydroxy salicylimide the least. Remaining showed the less repulsive effect as N- (p-talgyl) -2-hydroxy salicylimide > N- (p- chlorophenyle) -2 hydroxy salicylimide> N- (P-methoxy Phenyl-2 hydroxy salicylimide, respectively.

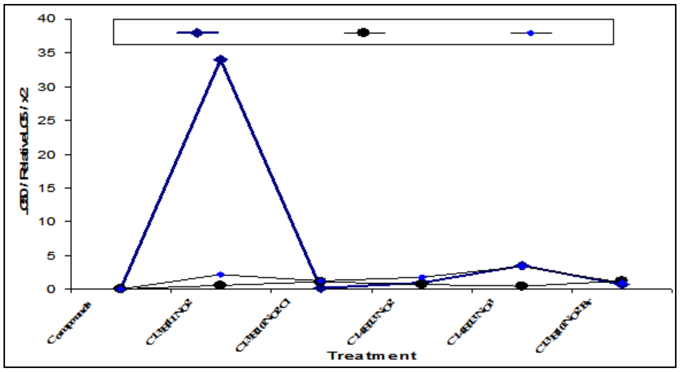


Fig 1: Repellent bioefficacy of Spodoptera litura due to amides of salicylic acid at 24 hrs

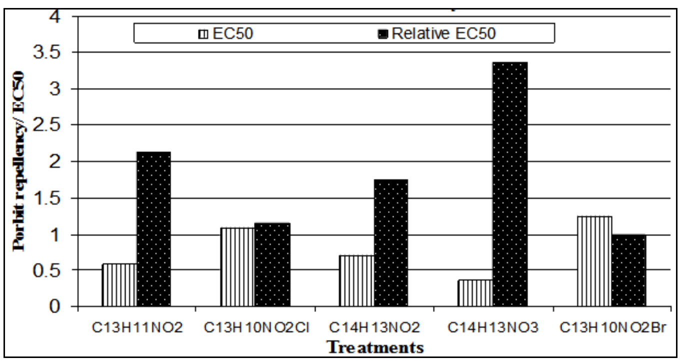


Fig 2: Log concentration probit repellency of callssobruchus chinensis due to amides of salicylic acid

5. Discussion

The present findings of repellent experiment are similar to those of Chandele, 2003, Chandel and Singh, 2017, Chandel, 2017 [20, 21, 22] who reported significant repellent biopotency of certain plant extract and their derivatives against pulse beetle, *Callosobruchus chinensis* Similarly, Jilani *et. al.* 1988, Jilani and Saxena, 1990 reported that promising repellent effects of turmeric oil, sweet flag oil, neem oil, and 'Margosan-O' are observed on red flour beetle and lesser grain borer, respectively [23, 24, 27]. Further, Schmutterer, 1988 and other workers reported that azadirachtin-containing and organic chemicals pesticides were also used as strong repellent and insecticides in the pest management [28, 29].

6. Conclusion

The findings of the present investigations indicate that botanical derivatives might be useful as insect control agents for commercial use. Among five amides, only N- (P-methoxy Phenyl-2 hydroxy Salicylimide (EC_{50}=0.37) showed highest repellency followed by N (phenyl) -2 hydroxy Salicylimide (EC_{50}=0.58), N- (p-tolyl) -2-hydroxy Salicylimide (EC_{50}=0.71) and N- (p- chlorophenyle) -2 hydroxysalicylimide (EC_{50}=1.08) against pulse beetle, *Callosobruchus chinensis*, where as N- (p- bromophenyle) -2 hydroxy salicylimide was proved the least repulsive to *C. chinensis* adults and taken as unit (1.00) against pulse beetle, *Callosobruchus chinensis*. All the extracts tested were effective to some degree of repellency reducing the feeding and destruction rates. More studies on major biochemical constituents of some plant derivatives are responsible for repellent activity to the test insect on mustard against larvae of *C. chinensis*. Need to be investigated.

7. Acknowledgement

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