

# INVESTIGATION OF INSECTICIDAL ACTIVITY OF SOME $\alpha,\beta$ -UNSATURATED CARBONYL COMPOUNDS AND THEIR SYNERGISTIC COMBINATION WITH NATURAL PRODUCTS AGAINST *PHENACOCCLUS SOLENOPSIS* TINSLEY

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**Abstract:** In continuation of our previous work, the current study explores an environmentally benign approach for the control of *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) using a synergistic combination of  $\alpha,\beta$ -unsaturated carbonyl compounds (1a-1e; 2a-2i) and the natural products, neem oil (N1) and nicotinic acid (N2). Our approach also evaluates the insecticidal activity of 3-(5-chloro-1,3-diaryl-1H-pyrazol-4-yl)-1-arylprop-2-en-1-one (2j-2o) consisting of bioactive moieties, viz., chalcone and pyrazole, in a single molecular structure. Compounds 2l and 2o exhibited maximum activity with 58% and 50% of mortality, respectively, under laboratory conditions. Among various test combinations, 2a-N2 showed maximum insecticidal activity, with 54% mortality, comparable to that of the most active newly synthesized compound, 2l, followed by 1c-N1 and 2g-N2 with 52% mortality. The compound 2a was also found to be non-toxic to potato tuber used as a plant substrate in the current investigation.

**Key words:** insecticidal, *Solanum* mealy bug, chalcones, pyrazole, neem oil, nicotinic acid

## INTRODUCTION

The *Solanum* mealy bug (SMB) or *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) also known as cotton mealy bug is one of the most devastating of the sap sucking polyphagous pests. The insect was noted for the first time in USA in 1898, and then its distribution subsequently spread to Canada, Latin America, the Caribbean in Western Hemisphere, South Africa, Zimbabwe, the Hawaiian Islands, Italy, Taiwan, Kiribati, the Gilbert Islands, Sicily, Israel and very recently to India (Chen *et al.* 2002; Santa *et al.* 2002; Dov 2005; Gautam *et al.* 2007; Akintola and Ande 2008). Among various host plants, cotton, eggplant, and sunflower are the most preferred food plants for this mealy bug. This bug can damage up to 80% of crops (Nalwar *et al.* 2009). The main difficulty in managing the SMB is their high reproductive potential, and their ability to survive under extreme weather conditions. The mealy waxy coating on its body also makes it difficult to manage this bug.

Current management of these insects is mainly based on spraying with organophosphorous and organochlorine pesticides like chlorpyrifos, monocrotophos and endosulfan (Fig. 1a, b, c).

It is well documented that continuous use of these pesticides have created problem of resistance, and resurgence, and harmful impact on the environment as well as

on humans. The replacement of toxic chemicals with compatible, environmentally friendly natural products, have their own limitations viz., less potency, lesser availability, structural complexity and instability. This necessitates the development of newer approaches to control these kinds of pests. For this reason, the current investigation is focused on (i) fabrication of compounds incorporating various bioactive fragments in a single molecule to improve bioactivity and minimize possibilities of resistance, and (ii) a broad spectrum screening of already existing bioactive compounds in combination with natural products, to reduce the concentration of synthetic compounds and natural products, while preserving and/or improving the efficacy of the products.

Our previous studies have established the broad spectrum biological activities of  $\alpha,\beta$ -unsaturated carbonyl compounds (1a-1e and 2a-2i) in combination with natural products viz., neem (*Azadirachta indica*) oil (N1), and nicotinic acid (N2). These combinations are known for their wide range of application in agriculture and pharmacology (Jermy 1990; Devkumar and Sukhdev 1993; Bodor and Offermanns 2008; Gupta *et al.* 2008), against human pathogenic bacteria, and plant pathogenic fungi (Rani *et al.* 2009a; Rani *et al.* 2009b). We extended our work to evaluate these synergistic combinations for insecticidal activity against *P. solenopsis*.

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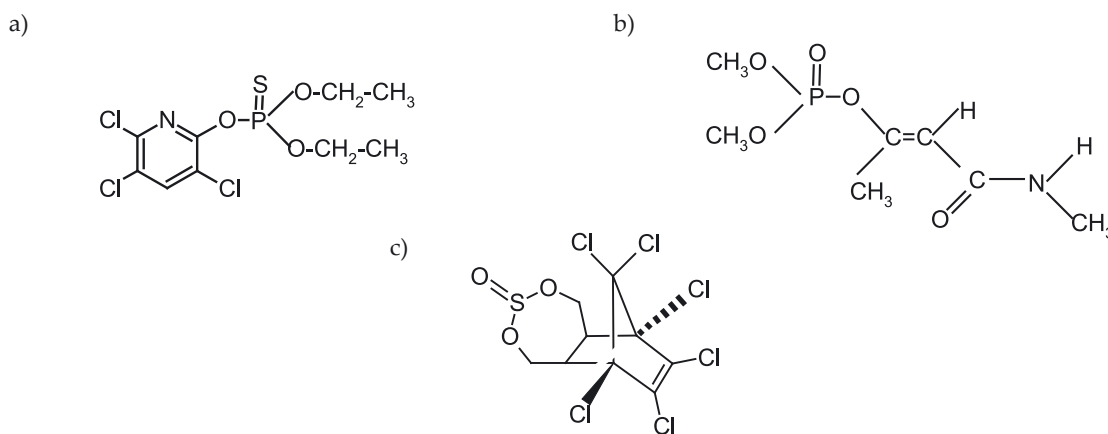


Fig. 1. a) Chlorpyrifos; b) Monocrotophos; c) Endosulfan

At the same time, chalcone derivatives containing pyrazole nucleus (2j-2o) were synthesized and screened against *P. solenopsis*.

These moieties are well documented for their multifaceted biological activities *viz.*, insecticidal (Dimmock *et al.* 1999; Shiga *et al.* 2003), antifungal (Akbas and Berber 2005; Lahtchev *et al.* 2008), antioxidant (Anto *et al.* 1995; Bhat *et al.* 2005; Arty *et al.* 2000), tyrosinase inhibitory (Dominguez *et al.* 2005), antimetabolic (Boumendjel *et al.* 2008), and antibacterial activity (Sivakumar *et al.* 2007; Rani and Jain 2008; Liu *et al.* 2008).

## MATERIALS AND METHODS

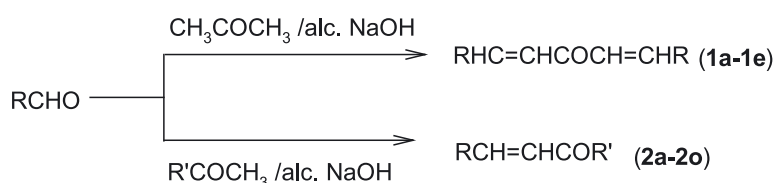
### Synthesis

All of the reported structural modifications were carried out by classical aldol condensation involving base-catalyzed condensation of the desired carbonyl com-

pounds followed by dehydration forming  $\alpha,\beta$ -unsaturated carbonyl compounds. The detailed synthetic route is described below.

In the case of compounds 2j-2o, the desired aldehyde was obtained by following the reported method (Gupta *et al.* 2009).


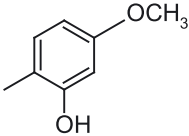




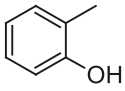

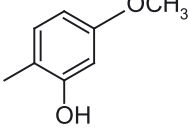


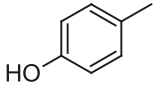
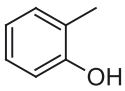
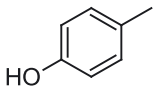

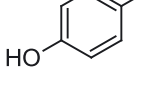

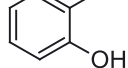
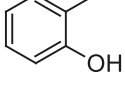
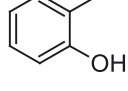
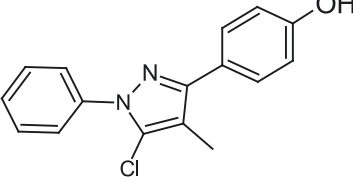

In a two-necked round-bottomed flask, equipped with a mechanical stirrer, a solution of the desired aldehyde (0.01 mol), was added to the required amount of alcoholic NaOH. Acetone (0.02 mol for 1a-1e) or the requisite aldehyde (0.01 mol for 2a-2o) was added slowly from a dropping funnel while being vigorously shaken. The reaction mixture was maintained at 25°C. The reaction was completed in 40-45 min with the formation of yellow precipitate. If no precipitation occurred, the reaction mixture was kept in a refrigerator overnight. The precipitate was filtered out and recrystallized using an appropriate solvent (Furniss *et al.* 2005) (Table 1).

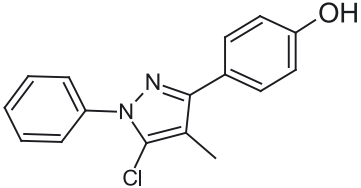
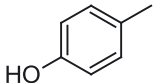
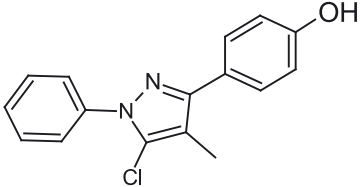
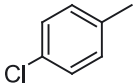
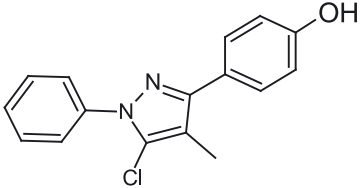
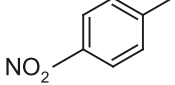
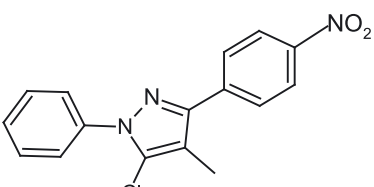
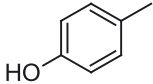
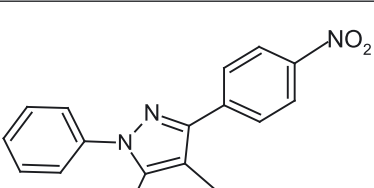
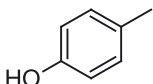


Scheme 1.

Table 1. Tested compounds

RHC=CHCOCH=CHR (1a-1e)			
Compound	R	R'	Compound name
1	2	3	4
1a		-	1,5-Diphenyl pent-1,4-diene-3-one
1b		-	1,9-Diphenylnon-1,3,6,8-tetraene-5-one
1c		-	1,5-bis(2-hydroxyphenyl)pent-1,4-diene-3-one

1	2	3	4
1d		-	1,5-Difurylpent-1,4-diene-3-one
1e		-	1,3-Bis(2-hydroxy,4-methoxyphenyl) prop-2-ene-1-one
RCH=CHCOR' (2a-2o)			
2a			1,3-di(phenyl)prop-2-ene-1-one
2b			1-furyl-3-phenylprop-2-ene-1-one
2c			1-phenyl, 3-(2-hydroxyphenyl) prop-2-ene-1-one
2d			1-phenyl-3(2-hydroxy-4-methoxy phenyl) prop-2-ene-1-one
2e			1-(4-hydroxyphenyl) 3-phenylprop-2-ene-1-one
2f			1-(4-hydroxyphenyl), 3-(2-hydroxyphenyl) prop-2-ene-1-one
2g			3-furyl-1-(4-hydroxyphenyl) prop-2-ene-1-one
2h			1-(2-hydroxyphenyl) 3-phenylprop-2-ene-1-one
2i			1,3 Bis -(2-hydroxyphenyl) prop-2-ene-1-one
2j			3-[5-chloro-3-(4-hydroxyphenyl)-1-phenyl-1-H-pyrazol-4-yl]-1-phenylprop-2-en-1-one

1	2	3	4
2k			3-[5-chloro-3-(4-hydroxyphenyl)-1-phenyl-1-H-pyrazol-4-yl]-1-(4-hydroxyphenyl)prop-2-en-1-one
2l			3-[5-chloro-3-(4-hydroxyphenyl)-1-phenyl-1-H-pyrazol-4-yl]-1-(4-chlorophenyl)prop-2-en-1-one
2m			3-[5-chloro-3-(4-hydroxyphenyl)-1-phenyl-1-H-pyrazol-4-yl]-1-(4-nitrophenyl)prop-2-en-1-one
2n			3-[5-chloro-3-(4-chlorophenyl)-1-phenyl-1-H-pyrazol-4-yl]-1-(4-hydroxyphenyl)prop-2-en-1-one
2o			3-[5-chloro-3-(4-nitrophenyl)-1-phenyl-1-H-pyrazol-4-yl]-1-(4-hydroxyphenyl)prop-2-en-1-one

### Characterization

InfraRed Spectra were recorded on Perkin Elmer BX-II Spectrophotometer using KBr pellets.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ) analysis was done with Model-Bruker ACP 300 and C H N analysis was done with Element analysensysteme GmbH VarioEL.

### Insecticidal activity: determination of percentage of mortality

#### Preparation of test samples

A stock solution of each compound was prepared in acetone. The combination of bioactive compounds (1a, 2a, 2c, 2g) with natural products, neem oil (*A. indica*) (N1) which is available commercially in Ayurvedic medical stores without a prescription in India, and nicotinic acid (N2) purchased from CDH, 044069 were prepared in a 1:1 ratio.

#### Bioassay

The nucleus culture of *P. solenopsis* was sourced from the biological control laboratory, Division of Entomology, Indian Agricultural Research Institute (IARI), New Delhi. The mealy bugs were reared on sprouted potato tuber

at a temperature of  $27\pm 2^\circ\text{C}$  with a relative humidity at  $60\pm 5\%$  following the method described by Gautam 2008a.

Fresh pieces of sprouted potato tuber (2.5 cm x 2.5 cm) were taken in Petri plates. The desired dose of compounds (1000 ppm) dissolved in acetone, were sprayed on the tuber pieces and air dried to evaporate the acetone. Initially adults of *P. solenopsis* were treated, but no significant effect was observed on them as shown in figure 2 a and b, the treated females even laid young once. Henceforth, experiments were carried out on third instar larva nymphs.

With the help of a brush, a total of 10 third-instar nymphs were released on each healthy sprout of tuber piece for direct contact. Each treatment, including the control, was replicated 5 times. The insects used in the experiments were examined for mortality after 24, 48 and 72 hours of treatment while being viewed under binocular (LEICA EZ4-D), Division of Entomology, IARI, New Delhi. Percentage of Mortality was determined using the following formula:

Percentage of mortality =  $(\text{Total No. of Insects introduced} - \text{No. of insects alive after treatment}) \times 100 / \text{Total No. of insects introduced}$ .

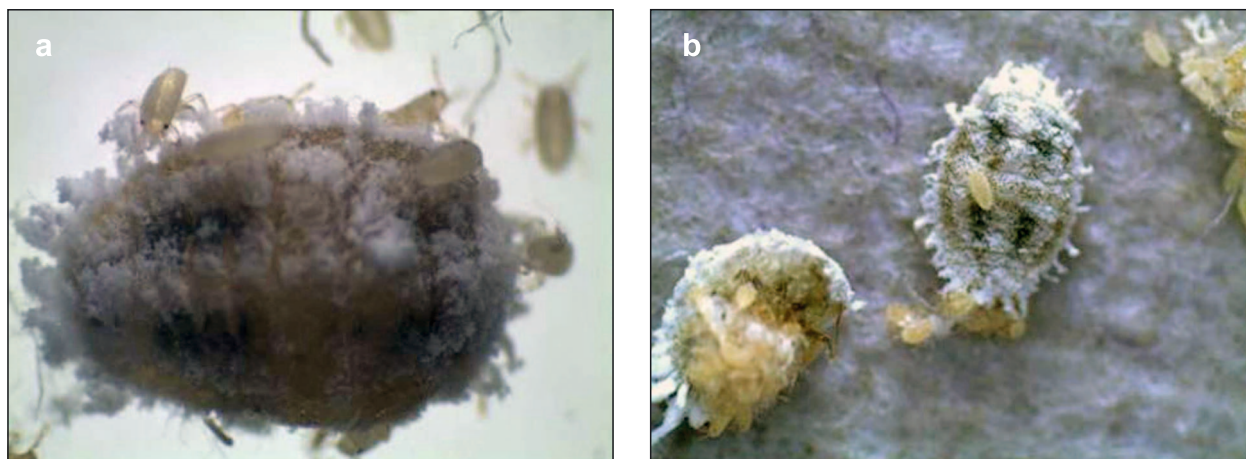


Fig. 2a, b. The adults remain unaffected after 72 hours of treatment of compounds

## RESULTS

### Characterization: compounds 2j-2o

The IR spectrum of all compounds showed characteristic bands at 2,900–3,100 and 1,610–1,570  $\text{cm}^{-1}$  due to aromatic ring skeleton vibration. The shift in wave number from a normal range of  $>\text{C}=\text{O}$  band of 1,700  $\text{cm}^{-1}$  to a range of 1,689–1,650  $\text{cm}^{-1}$ , confirmed the presence of a conjugated carbonyl group. A band in the range of

2,900–3,100  $\text{cm}^{-1}$  confirmed  $-\text{CH}=\text{CH}-$  skeleton. Bands near 778 and 696  $\text{cm}^{-1}$  signified the presence of monosubstituted benzene. The presence of  $-\text{OH}$  group was confirmed by a band near 3,355  $\text{cm}^{-1}$  and in 2m and 2o the band near 1,440  $\text{cm}^{-1}$  corresponded to the nitro group.  $^1\text{H}$  NMR and CHN results were found in accordance with their expected values (Table 2).

Table 2. Physical properties of compounds (2j–2o)

Compound	Melting point [°C]	Molecular formula	%C Observed (calculated)	%H Observed (calculated)	%N Observed (calculated)	Yield [%]	Rf
2j	116–118	$\text{C}_{24}\text{H}_{17}\text{ClN}_2\text{O}_2$	72.01 (71.91)	4.10 (4.27)	7.26 (6.99)	60	0.7
2k	160	$\text{C}_{24}\text{H}_{17}\text{ClN}_2\text{O}_3$	69.45 (69.15)	4.17 (4.11)	6.56 (6.72)	72	0.4
2l	110	$\text{C}_{24}\text{H}_{16}\text{Cl}_2\text{N}_2\text{O}_2$	66.18 (66.22)	3.77 (3.70)	6.35 (6.44)	69	0.9
2m	166	$\text{C}_{24}\text{H}_{16}\text{ClN}_3\text{O}_4$	65.32 (64.65)	3.65 (3.62)	9.35 (9.42)	73	0.9
2n	160	$\text{C}_{24}\text{H}_{16}\text{Cl}_2\text{N}_2\text{O}_2$	66.37 (66.22)	3.77 (3.70)	6.42 (6.44)	80	0.4
2o	200	$\text{C}_{24}\text{H}_{16}\text{ClN}_3\text{O}_4$	64.53 (64.65)	3.66 (3.62)	9.54 (9.42)	69	0.7

### Insecticidal activity

The tested samples showed appreciable activity against third instar nymph of *P. solenopsis* (Fig. 3a and b). Insecticidal activity after 24, 48 and 72 hours of treatment of all the compounds and combinations on *P. solenopsis* are shown in table 3 and 4, respectively. After 24 h only four compounds 1c, 2g, 2l and 2o showed slight activity. After 48 h of treatment, activity of the aforesaid compounds had increased. Three more compounds 2c, 2j, 2k, also demonstrated mild activity.

After 72 h, the activity of compounds 2l and 2o became appreciable with 58 and 50% of mortality, respectively. Similarly the activities of 2c, 2g and 2j, also became

mentionable with the percentage of mortality in the range of 34–38%.

The natural products N1 and N2 showed 36 and 38% of mortality, respectively, when tested alone (Table 4). Among all combinations of N1 with compounds exhibiting insecticidal activity when tested alone (1a–1e; 2a–2i), a good synergistic influence was observed. Moderately active 1c and 2a (Fig. 4a, b), increased their activity almost 1.5 times. An increase in activity of 2g from 34 to 44% was also observed due to the presence of N1. Similarly, the insecticidal activity of 2a and 2g was increased almost 1.5 times by N2 (Fig. 4b, c).

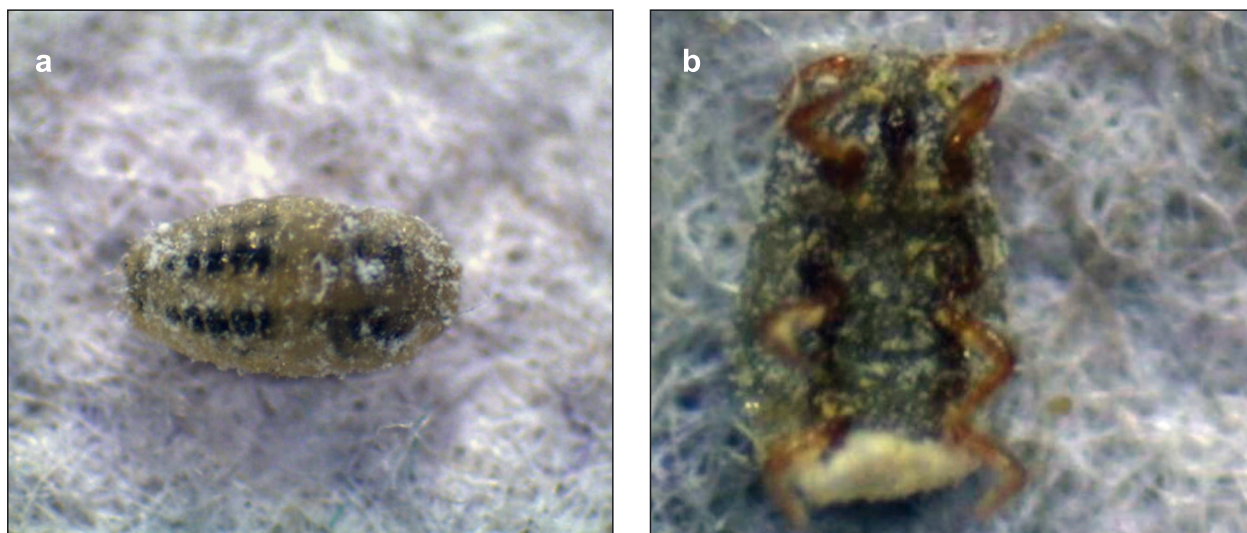


Fig. 3a and b. Effect of treatments on third instar nymph after 72 hours

Table 3. Insecticidal activity of synthetic compounds in terms of percentage of mortality

No.	Treatments	Percentage of mortality [%]			
		first observation (after 24 h)	second observation (after 48 h)	third observation (after 72 h)	total
1	1a	0.	0	0	0±0
2	1b	0	0	0	0±0
3	1c	10	10	10	30±4.082
4	1d	0	0	0	0±0
5	1e	0	0	0	0±0
6	2a	0	0	28	28±2.581
7	2b	0	0	0	0±0
8	2c	0	20	18	38±2.581
9	2d	0	0	0	0±0
10	2e	0	0	0	0±0
11	2f	0	0	0	0±0
12	2g	14	6	14	34±3.158
13	2h	0	0	0	0±0
14	2i	0	0	0	0±0
15	2j	0	20	14	34±3.158
16	2k	0	14	12	26±2.581
17	2l	18	14	26	58±2.581
18	2m	0	0	0	0±0
19	2n	0	0	0	0±0
20	2o	12	16	22	50±0

SEm ±0.892, CD (0.01%) is 3.32

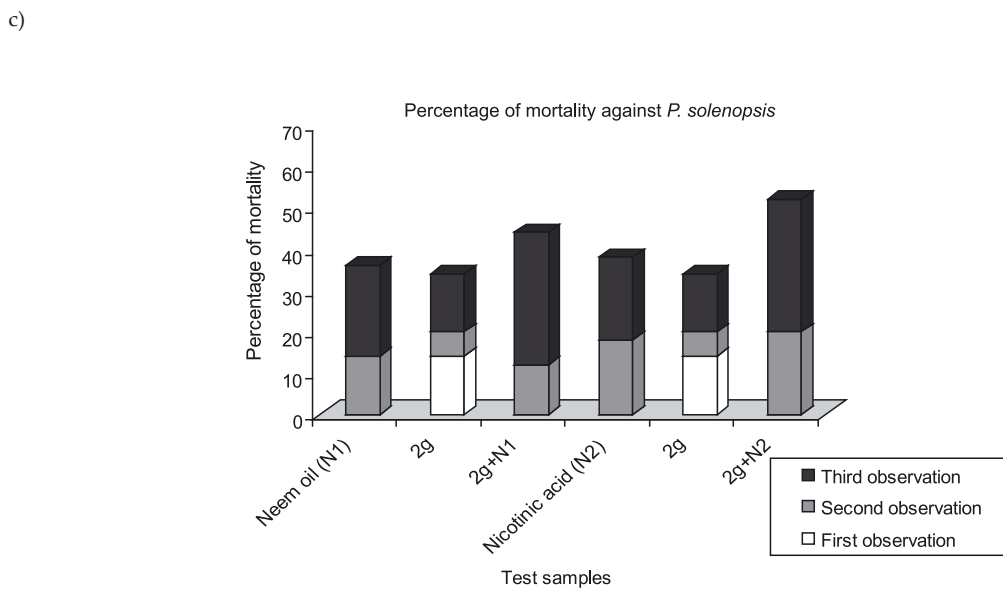
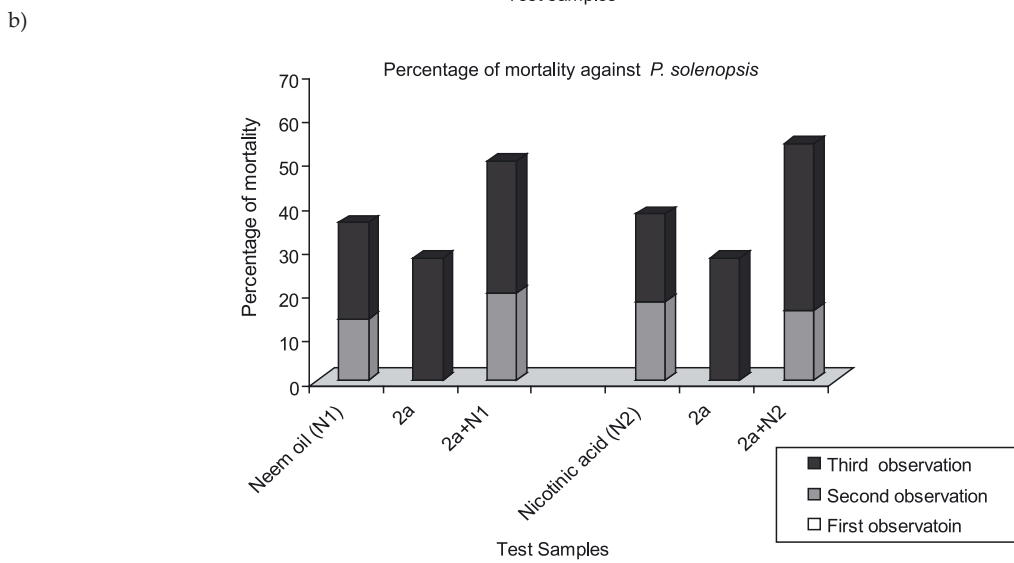
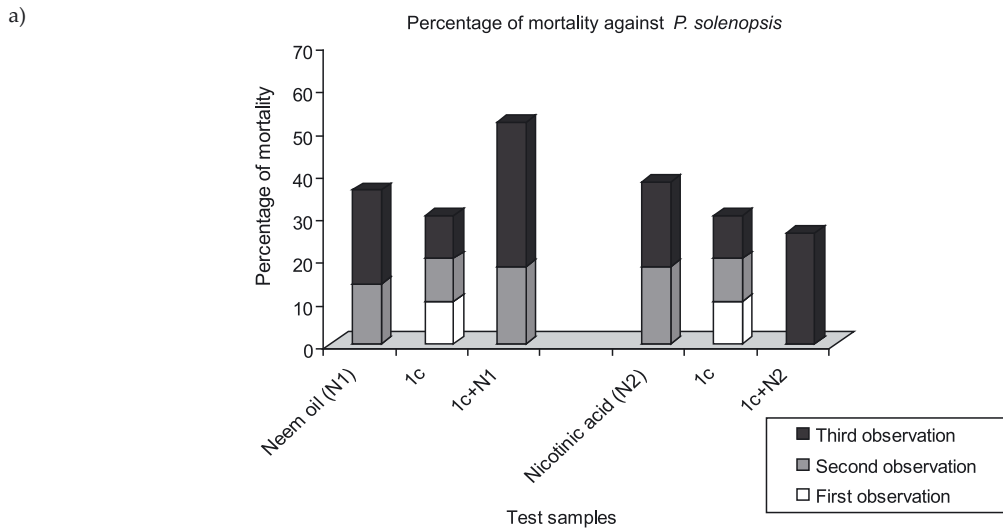


Fig. 4a, c. Graphs representing the percentage of mortality of compounds in combination with natural products

Table 4. Insecticidal activity of natural products and their combinations with bioactive compounds in terms of percentage of mortality

No.	Treatments	Percentage of mortality (%)			
		first observation (after 24 h)	second observation (after 48 h)	third observation (after 72 h)	total
1	neem oil (N1)	0	14	22	36±3.158
2	nicotinic acid (N2)	0	18	20	38±2.581
3	1c-N1	0	18	34	52±2.581
4	2a-N1	0	20	30	50±0
5	2c-N1	0	0	20	20±0
6	2g-N1	0	12	32	44±3.158
7	1c-N2	0	0	26	26±3.158
8	2a-N2	0	16	38	54±3.158
9	2c-N2	0	0	10	10±0
10	2g-N2	0	20	32	52±2.581

SEm ±1.15, CD (0.01%) is 4.42

## DISCUSSION

Solanum mealy bug is an emerging pest problem and its host-range is expanding day by day (Gautam 2008b). It has the potential for spreading aggressively and causing devastating effects on crops. This may result in a reluctance on the part of farmers to grow certain crops like cotton and eggplant which are the preferred food plants for SMB. After the realization of adverse effects on the environment and humans associated with the use of conventional pesticides, the current binary approach was undertaken to explore a new path for the control of SMB. This new direction investigates the insecticidal potential of compounds. Such an approach is designed to incorporate well known bioactive structures, viz., chalcones and pyrazole, in a single molecule, and insecticidal screening of a combination of synthetic compounds and natural products, known for their various biological activities.

Among various newly designed compounds, (2j–2o) 2l and 2o have shown considerable activity against the mealy bug. A reasonable structure relative interpretation

can be extracted by the fact that presence of a hydroxyl group at para position of benzene ring C (Fig. 5) and chloro group at para position of ring A, play an important role in the development of the activity in compound 2l, because removal or substitution of the chloro group with the hydroxy group diminishes the insecticidal activity (2j and 2k). Even swapping the chloro and the hydroxyl group completely inhibits the insecticidal activity of the resulting (Fig. 5) compound (2n). On the other hand, a reverse effect was observed with nitro and hydroxyl groups at these positions. In inactive compound 2m, the hydroxyl group is present at para position of ring C and nitro group at ring A, while in the active compound 2o, the hydroxyl group is at A ring and nitro group is at C ring. All these findings provide an insight for the designing of new and effective insecticides.

Our previous studies have established the promising antimicrobial activity of  $\alpha,\beta$ -unsaturated carbonyl compounds in combination with natural products, neem oil and nicotinic acid (Rani *et al.* 2009a; Rani *et al.* 2009b). The next step was to explore the insecticidal efficacy of these

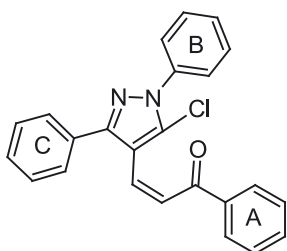


Fig. 5. Basic nucleus of compounds (2j–2o), representing the ring A, B, C

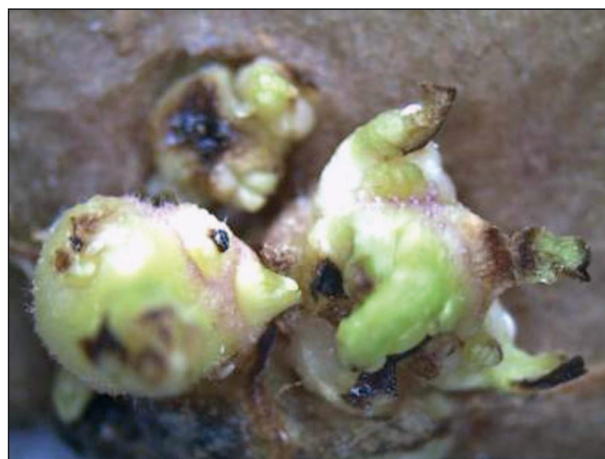


Fig. 6. Potato tuber after a 72 hour 2a test compound treatment



products. Good results were obtained – both of the natural products have shown good synergistic effect on insecticidal activity of 2a. Moreover, the insecticidal activity of combinations of mildly active synthetic compounds and natural products (2a-N1 and 2a-N2) was comparable to the activity of newly synthesized multi-structure containing compounds (2j–2o). It is worth mentioning, that compound 2a may be considered as ecologically safe as it can be synthesized by a simple and straightforward reaction without using harmful chemicals as solvent or catalyst. Compound 2a showed no toxicity on the plant substrate; the potato tuber remained green and healthy even after 72 h of treatment with the compound (Fig. 6). Also, the in vitro cell cytotoxicity of compounds with significant insecticidal activity, in combination, was investigated using the haemolytic assay (Bisht *et al.* 2007). The compounds exhibited negligible toxicity (7–14%) even up to high experimental concentrations *i.e.* 1000 ppm (Fig. 7) (Rani *et al.* 2010).

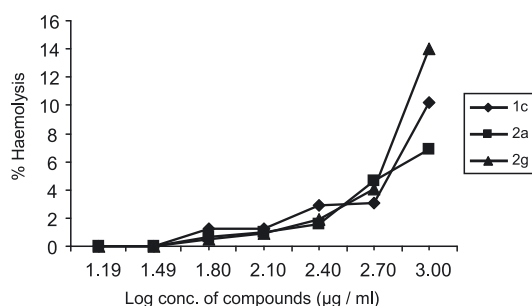


Fig. 7. Haemolytic activities of tested bioactive compounds

Based on such finding we may conclude that limited use of simple and safer chemicals in combination with natural products, is a greener and effective mode of control of noxious pests like *P. solenopsis*. This is the first study of this kind that can lead us towards the development of a new class of environmentally benign insecticides.

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