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Binary Combination of *Carica papaya*, *Areca catechu* and *Myristica fragrans* with Piperonyl Butoxide / MGK-264 against Freshwater Snail *Lymnaea acuminata*

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Abstrak: Piperonil butoksida (PB) dan MGK-264 telah digunakan untuk mempertingkatkan ketoksikan komponen aktif iaitu papain, arekolina dan miristisin, masing-masing daripada tumbuhan *Carica papaya*, *Areca catechu* dan *Myristica fragrans*, terhadap siput vector *Lymnaea acuminata*. Suatu perhubungan bersandar masa dan dos telah diperhatikan untuk ketoksikan kombinasi-kombinasi ini. Kesan toksik molusisida daripada tumbuhan-tumbuhan ini dengan kombinasi synergis PB dan MGK-264 didapati lebih tinggi daripada kesan rawatan tanpa kombinasi. Synergism tahap paling tinggi diperhatikan apabila MGK-264 telah digunakan dengan kombinasi lateks *C. papaya* (penambahan 10.47 kali) dan PB dengan papain (penambahan 8.5 kali).

Kata kunci: Fasioliasis, *Lymnaea acuminata*, Molusisida Tumbuhan, Piperonil Butoksida, MGK-264

Abstract: Piperonyl butoxide (PB) and MGK-264 were used to enhance the toxicity of the active components papain, arecoline and myristicin from the plants *Carica papaya, Areca catechu* and *Myristica fragrans*, respectively, against the vector snail *Lymnaea acuminata*. A time- and dose-dependent relationship was observed for the toxicity of these combinations. The toxic effects of these plant-derived molluscicides in combination with the synergists PB and MGK-264 were several times higher than the effect of the individual treatments. The highest degree of synergism was observed when MGK-264 was used in combination with *C. papaya* latex (10.47-fold increase) and PB was used with papain (8.35-fold increase).

Keywords: Fascioliasis, *Lymnaea acuminata,* Plant Molluscicide, Piperonyl Butoxide, MGK-264

INTRODUCTION

The incidence of fascioliasis, a serious parasitic disease affecting domestic ruminants and humans, surpasses all zoonotic helminthes infections worldwide (Haridy *et al*. 2002). Human infection with fascioliasis has been sporadic; however, in the past three decades, clinical cases and outbreaks of the disease have been reported (Haseeb *et al*. 2002). According to a World Health Organization (WHO) report from 2007, infections with fascioliasis were limited to specific and typical geographical areas (endemiotopes). However, the disease has now spread throughout the world with increased reports of human cases of fascioliasis in Europe, the Americas and Oceania (where only *Fasciola hepatica*

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is transmitted) and Africa and Asia (where two species, *Fasciola hepatica* and *Fasciola gigantica* can be found). The snail *Lymnaea acuminata* is the intermediate host for the liver fluke *F. gigantica*, which is responsible for endemic fascioliasis in cattle in the northern part of India (Singh & Agarwal 1981; Agarwal & Singh 1988). An effective method to reduce the incidence of fascioliasis is to control the population of vector snails, thereby breaking the life cycle of the flukes (Jigyasu & Singh 2010; Agarwal & Singh 1988). The control of the vector snail population using molluscicides is a well-recognised method for the control of fascioliasis.

Molluscicides of plant origin are becoming increasingly popular because they are cheaper and safer than their synthetic counterparts, as well as being potentially biodegradable and ecofriendly (Singh *et al*. 1996; Hostettmann & Lea 1988; Marston & Hostettmann 1985). However, concerted efforts to formulate improved compounds are needed to render the molluscicides more potent against harmful snails and safer for the environment. It has been reported that *Carica papaya* seed, *C. papaya* latex, *Areca catechu* seed (Jaiswal & Singh 2008), *Myristica fragrans* seed (nutmeg) and *M. fragrans* aril (mace) (Jaiswal & Singh 2009) are potent molluscicides against the harmful snail *L. acuminata.* The active molluscicidal components in *C. papaya* (seed and latex), *A. catechu* seed and *M. fragrans* seed are papain, arecoline and myristicin, respectively. In this study, we determined the toxicity against snail *L. acuminata* of binary combinations of the plant-derived molluscicides along with their active components and the synergists piperonyl butoxide (PB) and MGK-264.

MATERIALS AND METHODS

Animal Samples

The adult *L. acuminata* snails (average size 2.25±0.30 cm in length) were collected from the ponds, pools and lakes of the Gorakhpur district. The snails either adhere to the ventral surface of the leaves of aquatic plants or lie freely around the vegetation near the water banks. The sails were acclimatised to laboratory conditions in glass aquariums containing dechlorinated tap water for 72 h.

Binary Combinations

For the determination of molluscicidal activity, binary combinations of crude powder from *C. papaya (*seed and latex), *A. catechu* seed, *M. fragrans* (seed and aril) and the active components papain, arecoline and myristicin, respectively, were mixed with the synergists piperonyl butoxide and MGK-264 at a ratio of 1:5.

Molluscicidal Activity

The toxicity experiment against *L. acuminata* was performed using the method described by Singh and Agarwal (1984). The 10 experimental animals were maintained in a glass aquarium, containing 3 litres of dechlorinated tap water. The snails were exposed to different preparations of the plant-derived molluscicides and their active components along with the synergists PB (α [2-(2

butoxyethoxy) ethoxy]-4, 5-methylenedioxy-2-propyltoluene) (Mc Laughlin Gormley King Co., Minneapolis, Minnesota, USA) and MGK-264 (N-octyl bicycloheptene dicarboximide) (Mc Laughlin Gormley King Co., Minneapolis, Minnesota, USA) (Table 1). The aquaria (six in total) were set up for each concentration. The mortality was recorded at 24 h intervals for up to 96 h. To avoid contamination of the aquarium water, the dead animals were removed at each observation time. The mortality of the animals was established by the contraction of body within the shell, and a lack of response to probing with a needle was used as evidence.

Table 1: Concentration of binary combinations (1:5) of plant products and their active components with PB/MGK-264.

Treatments	Concentration (mg/l)
C. papaya seed + PB	10, 15, 20, 40
C. papaya seed + MGK-264	10, 15, 20, 40
C. papaya latex + PB	7, 15, 20, 25
C. papaya latex + MGK-264	5, 10, 20, 25
Papain + PB	1, 3, 5, 7
Papain + MGK-264	1, 3, 5, 7
A. catechu seed + PB	7, 15, 20, 25
A. catechu seed + MGK-264	10, 15, 20, 25
Arecoline + PB	0.1, 0.3, 0.5, 0.7
Arecoline + MGK-264	0.09, 0.1, 0.3, 0.5
M. fragrans seed + PB	5, 10, 20, 30
M. fragrans seed + MGK-264	5, 10, 20, 30
M. fragrans aril + PB	10, 20, 30, 35
M. fragrans aril + MGK-264	5, 10, 20, 30
Myristicin + PB	0.5, 0.7, 0.9, 1.1
Myristicin + MGK-264	0.3, 0.5, 0.7, 0.9

The lethal concentration value (LC_{50}), upper and lower confidence limit (UCL and LCL) and slope values were calculated according to the Probit or Logit (POLO) computer programme (Robertson *et al*. 2007). The regression coefficient between the exposure time and the different LC_{50} values was determined (Sokal & Rohlf 1973).

Preparation of Crude Powder

The *C. papaya* seeds and latex were collected in the field from locally cultivated crops. The *A. catechu* seeds, commonly termed *Supari*, and the *M. fragrans* seed and aril were purchased from a local market in the Gorakhpur district (India).

The epidermis of the unripe fruit was cut, and the white milky latex of the *C. papaya* was drained into a graduated tube and lyophilised at –40°C. The lyophilised powder was stored in an airtight desiccator for future use. The seeds from the ripe *C. papaya* fruits were dried in an incubator at 37°C. The *C. papaya*

seeds, *A. catechu* seeds, and *M. fragrans* seeds and aril were pulverised separately using a grinder, and the crude powders obtained were used in the toxicity experiment (Jaiswal & Singh 2009; Jaiswal & Singh 2008).

Pure Compounds

The papain (cysteine protease), arecoline hydrobromide (methyl 1,2,5,6-tetrahydro-1-methyl-3-pyridine carboxylate hydrobromide) and myristicin [4-methoxy-6-(2-propenyl)-1,3-benzodioxole] were purchased from Sigma Chemical Co. (Saint Louis, Missouri, USA).

RESULTS

The combinations of the *C. papaya* seeds / latex, *A. catechu* seeds, *M. fragrans* seeds / aril and their pure compounds papain, arecoline, myristicin with the synergists PB and MGK-264 demonstrated that the molluscicidal activity of the mixtures against the snail *L. acuminata* was time- and dose-dependent. A significant (p<0.05) negative regression was observed between the exposure time and the LC_{50} of the mixtures (Tables 2–4).

At 24 h, the toxicity of the binary combination of the *C. papaya* seed powder with PB / MGK-264 against *L. acuminata* was 5.81- / 5.52-times higher than the treatment with the seed powder alone. Similarly, at 96 h, the toxicity of the C. *papaya* seed powder combined with PB / MGK-264 was 5.05- / 6.72-times higher than the treatment with the powder alone. The binary combination of the *C. papaya* latex with PB / MGK-264 at 24 h was 1.72- / 3.51-times more toxic than *C. papaya* latex alone, and after 96 h of exposure, the toxicity of the *C. papaya* latex plus PB / MGK-264 was enhanced 2.89- / 10.47-fold compared with the absence of the synergists. At 24 h, the binary combination of papain with PB / MGK-264 was 2.16- / 1.82-times more toxic than the treatment with papain alone. At 96 h, the toxicity of the papain with PB / MGK-264 was 8.35- / 7.80-times higher than the treatment with just papain. The 96-h toxicity of the *C. papaya* seed powder was more effective in combination with MGK-264 (LC_{50} 9.16 mg/l) than with PB (LC_{50} 12.19 mg/l). Similarly, the *C. papaya* latex was more effective in combination with MGK-264 (LC_{50} 0.80 mg/l) than with PB (LC_{50} 2.90 mg/l). The papain was more effective against *L. acuminata* in combination with PB (LC_{50} 1.17 mg/l) than with MGK-264 (LC_{50} 1.25 mg/l) (Table 2).

At 24 h, the binary combination of the *A. catechu* seed powder with PB / MGK-264 was 2.34- / 1.95-times more toxic against the snail *L. acuminata* than the treatment with the *A. catechu* seed powder alone, and after exposure for 96 h, the toxicity of the *A. catechu* seed powder in combination with PB / MGK-264 was increased 3.24- / 3.19-fold. The binary combination of the arecoline with PB / MGK-264 at 24 h was 0.35- / 1.03-times more toxic against *L. acuminata* than the treatment with arecoline alone, and at 96 h, the toxicity of the arecoline with PB / MGK-264 was increased 1.04- / 2.33-fold. The *A. catechu* seed powder was more toxic in combination with PB (LC_{50} 3.80 mg/l) than with MGK-264 (LC_{50} 3.86 mg/l). The arecoline plus MGK-264 (LC_{50} 0.06 mg/l) was more effective against *L. acuminata* than the arecoline plus PB (LC_{50} 0.13 mg/l) (Table 3).

At 24 h, the binary combination of the *M. fragrans* seed powder and PB / MGK-264 was 3.59- / 4.64-times more toxic against the snail *L. acuminata* than the treatment with *M. fragrans* seed powder alone, and at 96 h, the toxicity of the *M. fragrans* seed powder in combination with PB / MGK-264 increased 4.43- / 4.38-fold. At 24 h, the binary combination of the *M. fragrans* aril powder with PB / MGK-264 was 1.96- / 2.64-times more toxic than the treatment with *M. fragrans* aril powder alone, and after 96 h of exposure, the toxicity was enhanced 6.30- / 4.50-fold. At 24 h, the binary combination of the myristicin with PB / MGK-264 was 1.12- / 1.25-times more toxic than the treatment with myristicin alone. The *M. fragrans* aril powder was more effective in combination with PB (LC_{50} 4.54 mg/l) than with MGK-264 (LC_{50} 6.36 mg/l), and the myristicin was more effective in combination with MGK-264 (LC_{50} 0.41 mg/l) than with PB (LC_{50} 0.62 mg/l) (Table 4).

The slope values were steep, and the separate estimation of the LC_{50} based on each of the six replicates was within the 95% confidence limits of LC_{50}. The t ratio was greater than 1.96, the heterogeneity factor was less than 1.0, and the 'g' value was less than 0.5 at all probability (90, 95, 99) levels.

DISCUSSION

The toxicity study clearly demonstrated that the molluscicidal activity of the *C. papaya* seed and latex, *A. catechu* seed, *M. fragrans* seed and aril and the active components papain and arecoline was enhanced when combined with the synergists PB and MGK-264. The synergists PB and MGK-264 are typically used in combination with carbamate, organophosphate and pyrethroid pesticides (Rao & Singh 2001; Sahay *et al*. 1991; Casida 1970). The synergistic activity is produced mainly through the inhibition of the mixed function oxidase (MFO) activity, which detoxifies xenobiotics (Rao & Singh 2001; Matsumura 1985; Metcalf 1967) or possibly increases the penetration of the toxin, resulting in a high titre of the toxin at the active sites (Rao & Singh 2001).

The synergists PB and MGK-264 alone are not toxic to the snail *L. acuminata* (Sahay *et al*. 1991; Singh & Agarwal 1989). The activity of the binary mixture is non-interactive (Plackett & Hewlett 1952) because each component does not affect the transport and final concentration of the other compound at the site of action. In this case study, the aquatic environment was advantageous for the penetration of the toxicant because the snail body was bathed in a dilute solution of the toxicant. The maximum effect is obtained when the synergist penetrates the organism and is transported rapidly to the active site. It appears that the high level of synergism in snails may be because of the rapid penetration of synergists through the animal's soft foot.

The toxicity of the *C. papaya* seed in combination with PB and MGK-264 (96 h, LC_{50} 12.1 mg/l and 9.1 mg/l, respectively), *C. papaya* latex with PB and MGK-264 (96 h, LC_{50} 2.9 mg/l and 0.8 mg/l, respectively), *A. catechu* seed with PB and MGK-264 (96 h, LC_{50}-3.8 mg/l and 3.8 mg/l, respectively), *M. fragrans* seed with PB and MGK-264 (96 h, LC_{50} 8.3 mg/l and 8.4 mg/l, respectively), and *M. fragrans* aril with PB and MGK-264 (96 h, LC_{50} 4.5 mg/l and 6.3 mg/l,

respectively) against the snail *L. acuminata* was relatively high compared with the individual treatments, i.e., *C. papaya* seed (96 h, LC_{50} 61.5 mg/l), *C. papaya* latex (96 h, LC_{50} 8.3 mg/l), *A. catechu* seed (96 h, LC_{50}-12.3 mg/l) (Jaiswal & Singh 2008), *M. fragrans* seed (96 h, LC_{50} 36.9 mg/l), and *M. fragrans* aril (96 h, LC_{50} 28.6 mg/l) (Jaiswal & Singh 2009). The papain and arecoline treatments in combination with PB / MGK-264 were also more toxic than the treatments with papain and arecoline alone. The data indicated that the mixed function oxidase (MFO) was inhibited by the PB and MGK-264 (Rao & Singh 2001; Matsumura 1985; Metcalf 1967), suggesting that the molluscicides were not detoxified or that their penetration to the target site was increased inside the snail body (Singh & Singh 2003). The *C. papaya* latex plus MGK-264 (10.47-fold increase in toxicity), papain plus PB (8.35-fold increase in toxicity) and papain plus MGK-264 (7.80-fold increase in toxicity) demonstrated the highest degrees of synergism. In contrast, the myristicin in combination with PB / MGK-264 did not demonstrate significant synergistic activity. In a previous study, myristicin was identified as a molluscicidal component (Jaiswal & Singh 2009); however, the lack of a synergistic response in combination with PB / MGK-264 indicated that the toxicity of myristicin was not influenced by the mixed function oxidase. After the 96 h exposure period, a small amount of antagonistic activity was observed (the synergistic ratio was less than 1.0), indicating that myristicin, PB and MGK-264 may act at the same site (possibly on MFOs); therefore, the toxicity of myristicin was not synergised by PB or MGK-264 (Singh *et al.* 2010).

The steep slope indicated that even small increases in the concentration of the compounds caused high snail mortality. The t-ratio values higher than 1.96 indicated that the regression was significant. The heterogeneity factor values were less than 1.0, indicating that in the replicate tests of random samples, the concentration response curves fell within the 95% confidence limits; therefore, the model fits the data adequately. Because the index of significance of potency estimation (g-value) was less than 0.5, the mean was within the limits at all probabilities (90, 95, 99).

Table 2: Toxicity against *L. acuminata* of binary combinations of *C. papaya* (seed and latex) and papain with MGK-264 and PB.

Exposure (h)	Treatments	Toxicity LC_{50} (LCL–UCL) (mg/l)	Synergistic ratio	Slope value
24	*CPS	127.26 (108.62–174.86)		3.36±0.69
	CPS + PB	21.91 (18.63–31.07)	5.81	3.48±0.76
	CPS + MGK	23.07 (19.18–35.67)	5.52	3.23±0.75
	*CPL	19.92 (16.90–25.99)		2.94±0.50
	CPL + PB	11.61 (9.01–21.44)	1.72	2.33±0.56
	CPL + MGK	5.67 (3.42–22.32)	3.51	1.33±0.34
	*Papain	16.63 (14.55–21.55)		4.46±0.86
	Papain + PB	7.69 (5.24–18.15)	2.16	1.16±0.29
	Papain + MGK	9.15 (6.67–17.22)	1.82	1.71±0.35

(*continued on next page*)

Table 2: (*continued*)

Exposure (h)	Treatments	Toxicity LC_{50} (LCL–UCL) (mg/l)	Synergistic ratio	Slope value
48	*CPS	102.87 (89.23–132.50)		2.82±0.62
	CPS + PB	16.57 (14.77–19.87)	6.21	3.42±0.69
	CPS + MGK	17.52 (15.19–23.15)	5.87	2.83±0.68
	*CPL	16.52 (13.68–22.14)		2.14±0.41
	CPL + PB	7.65 (6.37–10.61)	2.16	2.31±0.49
	CPL + MGK	4.21 (2.51–22.50)	3.92	0.99±0.30
	*Papain	15.07 (13.14–19.75)		3.50±0.74
	Papain + PB	4.91 (3.23–11.05)	3.07	0.89±0.26
	Papain + MGK	7.43 (5.06–17.44)	2.03	1.13±0.28
72	*CPS	80.93 (68.62–97.03)		2.46±0.60
	CPS + PB	14.36 (12.65–16.75)	5.64	3.05±0.67
	CPS + MGK	13.15 (10.97–15.55)	6.15	2.50±0.66
	*CPL	11.86 (9.78–14.61)		2.05±0.39
	CPL + PB	3.33 (1.94–4.22)	3.56	1.89±0.47
	CPL + MGK	2.01 (1.47–3.33)	5.90	1.23±0.29
	*Papain	12.74 (11.30–15.51)		3.27±0.69
	Papain + PB	2.71 (1.46–4.26)	4.70	0.88±0.26
	Papain + MGK	3.17 (2.23–4.43)	4.02	1.21±0.27
96	*CPS	61.56 (51.79–69.03)		3.53±0.64
	CPS + PB	12.19 (10.01–14.06)	5.05	2.67±0.66
	CPS + MGK	9.16 (6.75–10.62)	6.72	3.19±0.71
	*CPL	8.38 (6.61–9.98)		2.23±0.39
	CPL + PB	2.90 (2.02–3.52)	2.89	2.86±0.53
	CPL + MGK	0.80 (0.54–1.03)	10.47	1.56±0.29
	*Papain	9.74 (8.65–10.81)		3.71±0.69
	Papain + PB	1.17 (0.27–1.93)	8.35	0.88±0.26
	Papain + MGK	1.25 (0.65–1.77)	7.80	1.36±0.27

Notes: LCL, lower confidence limit; UCL, upper confidence limit; +, linear regression between log x and log y; ++, non-linear regression between log x and log y; CPS, *C. papaya* seed; CPL, *C. papaya* latex. The snails (6 batches, each containing 10 snails) were exposed to the different concentrations of the treatments. The mortality was recorded every 24 h. The concentrations represent the final concentrations (w/v) in the glass aquarium water. The t-ratio was 1.96, heterogeneity factor < 1.0 and g value < 0.5 at all probability levels. The slope value is reported as the mean±SE. A significant negative regression (p<0.05) was observed between the exposure time and the LC_{50} of the treatments. ts, testing significance of the regression coefficient of CPS + PB = –18.61++; CPL + PB = –5.25; papain + PB = –11.09+; CPS + MGK = –18.21+; CPL + MGK = –13.07+; and papain + MGK = –8.06+.
*Jaiswal and Singh (2008)

Table 3: Toxicity against *L. acuminata* of binary combinations of *A. catechu* and arecoline with MGK-264 and PB.

Exposure (h)	Treatments	Toxicity LC_{50} (LCL–UCL) (mg/l)	Synergistic ratio	Slope value
24	*A	27.23 (23.74–33.87)		3.46 ± 0.58
	A + PB	11.63 (9.18–20.02)	2.34	2.61 ± 0.59
	A + MGK	13.99 (9.82–44.14)	1.95	1.88 ± 0.54
	*Arecoline	0.49 (0.38–0.80)		2.33 ± 0.49
	Arecoline + PB	1.40 (0.85–5.22)	0.35	1.37 ± 0.35
	Arecoline + MGK	0.48 (0.31–1.28)	1.03	1.06 ± 0.27
48	*A	23.32 (20.56–27.88)		3.27 ± 0.53
	A + PB	8.93 (7.26–13.83)	2.61	2.13 ± 0.50
	A + MGK	8.79 (7.21–13.10)	2.65	2.22 ± 0.51
	*Arecoline	0.34 (0.28-0.46)		2.05 ± 0.41
	Arecoline + PB	0.89 (0.57–3.01)	0.38	1.03 ± 0.28
	Arecoline + MGK	0.31 (0.21–0.67)	1.10	0.95 ± 0.26
72	*A	17.45 (15.38–19.82)		3.21 ± 0.51
	A + PB	6.10 (4.97–7.88)	2.86	1.91 ± 0.47
	A + MGK	4.72 (3.89–5.47)	3.70	2.58 ± 0.48
	*Arecoline	0.21 (0.17–0.27)		1.74 ± 0.38
	Arecoline + PB	0.51 (0.33–1.18)	0.42	0.90 ± 0.26
	Arecoline + MGK	0.10 (0.04-0.14)	2.16	1.06 ± 0.27
96	*A	12.32 (9.70–14.32)		2.75 ± 0.52
	A + PB	3.80 (2.92–4.45)	3.24	2.65 ± 0.49
	A + MGK	3.86 (3.06–4.49)	3.19	2.82 ± 0.50
	*Arecoline	0.14 (0.09–0.17)		1.79 ± 0.38
	Arecoline + PB	0.13 (0.04–0.21)	1.04	0.94 ± 0.26
	Arecoline + MGK	0.06 (0.02–0.09)	2.33	1.23 ± 0.29

Notes: LCL, lower confidence limit; UCL, upper confidence limit; +, linear regression between log x and log y; ++, non-linear regression between log x and log y; A, *A. catechu*. The snails, 6 batches each containing 10 snails, were exposed to the different concentrations of the treatments. The mortality was recorded every 24 h. The concentrations represent the final concentrations (w/v) in the glass aquarium water. The t-ratio was 1.96, heterogeneity factor < 1.0 and g value < 0.5 at all probability levels. The slope value is reported as the mean±SE. A significant negative regression (p<0.05) was observed between the exposure time and the LC_{50} of the treatments. ts, testing significance of the regression coefficient of A + PB = –33.48+; arecoline + PB = –18.60+; A + MGK = –7.94++; and arecoline + MGK = –5.79+.

*Jaiswal and Singh (2008)

Table 4: Toxicity against *L. acuminata* of binary combinations of *M. fragrans* (seed and aril) and myristicin with MGK-264 and PB.

Exposure (h)	Treatments	Toxicity LC_{50} (LCL-UCL) mg/l	Synergistic ratio	Slope value
24	**MFS	74.15 (64.19–91.64)		2.79 ± 0.51
	MFS + PB	20.68 (15.78–51.91)	3.59	2.63 ± 0.75
	MFS + MGK	15.98 (13.92–20.93)	4.64	3.90 ± 0.78
	**MFA	38.61 (36.46–42.22)		8.09 ±1.34
	MFA + PB	19.75 (13.52–58.98)	1.96	1.72 ± 0.46
	MFA + MGK	14.64 (11.94–23.72)	2.64	3.11 ± 0.72
	**Myristicin	1.51 (0.94–4.47)		1.35 ± 0.31
	Myristicin + PB	1.35 (1.12–2.13)	1.12	3.32 ± 0.79
	Myristicin + MGK	1.21 (0.93–2.25)	1.25	2.46 ± 0.59
48	**MFS	54.42 (45.29–64.76)		2.31 ± 0.48
	MFS + PB	14.43 (12.26–21.20)	3.77	2.63 ± 0.69
	MFS + MGK	13.68 (12.05–17.21)	3.98	3.22 ± 0.70
	**MFA	33.99 (32.18–36.29)		7.07 ± 1.17
	MFA + PB	10.52 (8.55–14.99)	3.23	1.93 ± 0.41
	MFA + MGK	12.51 (10.35–19.68)	2.72	2.57 ± 0.64
	**Myristicin	0.71 (0.50–1.32)		1.18 ± 0.26
	Myristicin + PB	1.19 (0.98–2.01)	0.60	2.51 ± 0.69
	Myristicin + MGK	0.88 (0.71–1.38)	0.81	2.06 ± 0.50
72	**MFS	45.99 (37.43–53.50)		2.52 ± 0.48
	MFS + PB	9.20 (8.04–10.20)	5.00	3.69 ± 0.70
	MFS + MGK	10.41 (9.17–11.84)	4.42	3.23 ± 0.68
	**MFA	30.22 (28.09–32.06)		6.47 ± 1.15
	MFA + PB	6.57 (5.19–8.13)	4.60	1.85 ± 0.38
	MFA + MGK	8.74 (7.57-10.61)	3.46	2.63 ± 0.61
	**Myristicin	0.37 (0.26–0.53)		1.16 ± 0.24
	Myristicin + PB	0.77 (0.66–0.89)	0.48	2.81 ± 0.66
	Myristicin + MGK	0.69 (0.57-0.95)	0.54	1.89 ± 0.48
96	**MFS	36.95 (27.74–43.75)		2.54 ± 0.49
	MFS + PB	8.35 (7.38–9.13)	4.43	4.63 ± 0.74
	MFS + MGK	8.43 (6.95–9.50)	4.38	3.26 ± 0.69
	**MFA	28.61 (26.67–30.15)		7.70 ± 1.20
	MFA + PB	4.54 (3.24–5.60)	6.30	1.96 ± 0.38
	MFA + MGK	6.36 (5.91–7.12)	4.50	3.52 ± 0.63

(*continued on next page*)

Table 4: (*continued*)

Exposure (h)	Treatments	Toxicity LC_{50} (LCL-UCL) mg/l	Synergistic ratio	Slope value
	**Myristicin	0.16 (0.10–0.21)		1.53 ± 0.26
	Myristicin + PB	0.62 (0.50–0.70)	0.26	3.14 ± 0.67
	Myristicin + MGK	0.41 (0.31–0.48)	0.39	2.41 ± 0.48

Notes: LCL, lower confidence limit; UCL, upper confidence limit; +, linear regression between log x and log y; ++, non-linear regression between log x and log y; MFS, *M. fragrans* seed; MFA, *M. fragrans* aril. The snails (6 batches, each containing 10 snails) were exposed to different concentrations of the treatments. The mortality was recorded every 24 h. The concentrations represent the final concentrations (w/v) in the glass aquarium water. The t-ratio was 1.96, heterogeneity factor < 1.0 and g value < 0.5 at all probability levels. The slope value is reported as the mean±SE. A significant negative regression (p<0.05) was observed between the exposure time and the LC_{50} of the treatments. ts, testing significance of the regression coefficient of MFS + PB = –8.06++; MFA + PB = –17.37++; myristicin + PB = –6.95+; MFS + MGK = –15.46+; MFA + MGK = –13.16+; and myristicin + MGK = –14.32+.

**Jaiswal and Singh (2009)

CONCLUSION

The data indicated that the synergists PB and MGK-264 could play an important role in the control of the vector snail population and ultimately fascioliasis. The synergists increased the efficiency of the plant-derived molluscicides, suggesting that the concentrations of these plant-derived molluscicides could be reduced, producing a lethal effect through synergism in areas of treated water where the concentration of the toxins is sub-lethal to the snail population. Effective toxic concentration of the compounds was lower when combined with the synergists than when used alone, which is safer for the aquatic environment.

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