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Short Communication

Evaluating the Impact of Benzimidazole Compounds on Microsporidial Infection in *Bombyx mori L.,* Specifically Lamerin Breed

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Six benzimidazole derivatives *viz.,* Metronidazole, Albendazole, Tinidazole, Ornidazole, Mebendazole and Satinidazole at three concentrations (0.25, 0.50 and 1.00%) were screened against microsporidiosis of Lamerin breed of the silkworm, *Bombyx mori in vitro* and *in vivo*. All drugs were found significantly effective in minimizing the microsporidiosis. Further more, mebendazole were found more effective at all concentrations and emerged as the most efficient and promising drug for minimizing the microsporidiosis to the significant extent in Lamerin breed of the silkworm, *B mori*.

Key words: Microsporidiosis, benzimidazole, *Bombyx mori*, lamerin breed.

INTRODUCTION

Chemotherapy was attempted by many researchers to control pebrine in insects in general and in silkworms in particular. Sahay et al. (2005), Hayasaka (1991), and Baig (1994), attempted to asses the effect of chemicals or drugs on microsporidians. Griyaghey (1976) studied the effect of chemotherapic agents on Pebrine of Tassar silkwom, *Antheraea mylitta* and found an effective way to control the microsporidiosis. Schmah and Benini (1998) reported the effectiveness of benzimidazole derivatives in controlling the microsporidiosis of fishes. The scientific literature shows there are no report as such on the effects of benzimidazole derivatives on the microsporidiosis of Lamerin breed of the silkworm, *B. mori*. So in the present study the effect of benzimidazole derivatives on the microsporidiosis of Lamerin breed is investigated.

MATERIALS AND METHODS

Six benzimidazole derivatives *viz.,* Metronidazole (M/s R.P.L. Pharmaceuticals Pvt. Ltd., New Delhi, India), Albendazole, (M/s Cipla Meditab Specialties Pvt. Ltd., Goa, India), Tinidazole, (M/s Kopran Pvt. Ltd. Khalapur), Ornidazole, (M/s Vapicare Pharma Pvt. Ltd. India), Mebendazole, (M/s Cipla Ltd. by Meditab Specialties Pvt. Ltd., Goa, India) and Satinidazole, (M/s Nicholas Piramal India Pvt. Ltd. Auragabad, India) were screened for antimicrosporidian activity against Lamerin microsporidian spores.

In vitro efficacy of benzimidazole derivative against microsporidia

These drugs were screened *in vitro* for anti microsporidian activity. The microsporidian spores (1×10^7 spores/ml) were suspended in one ml of drugs of concentrations *viz.,* 0.25, 0.50 and 1.00% for 30 min respectivelly. The incubated spores were centrifuged at 300 rpm for 5 min and the sediment was collected washed properly in distilled water by repeated centrifugation and were subjected to viability test.

Toxicity test of drugs

The drugs were screened for their toxicity at 1% dosage by feeding to 2nd instar silkworm larvae daily once for three days along with the mulberry leaves and the toxicity symptoms were observed up to 10 days.

In another set of experiment, one day old 3rd instar Lamerin

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Table 1. *In vitro* screening of different of benzimidazole derivatives against microsporidia.

S/No.	Name of the drug	Chemical formula	Concentrations (%)	Effective
1	Metronidazole	$C_6H_9N_3O_3$	0.25	-
			0.50	-
			1.00	+
2	Albendazole	$C_{12}H_{15}N_3O_2S$	0.25	-
			0.50	-
			1.00	+
3	Tinidazole	$C_8H_{13}N_3O_4S$	0.25	-
			0.50	-
			1.00	-
4	Ornidazole	$C_7H_{10}C_1N_3O_3$	0.25	-
			0.50	-
			1.00	+
5	Mebendazole	$C_{16}H_{13}N_3O_3$	0.25	+
			0.50	+
			1.00	+
6	Satranidazole	$C_{18}H_{29}C_1N_3$-H_3PO_4	0.25	-
			0.50	-
			1.00	-
			1.00	-
	Control		-	-

- Ineffective; + Effective.

silkworm larvae were classified into four batches. Each batch which consisted of three replications of 100 larvae was per orally inoculated with Lb_{ms} spores (1×10^7 spores/ml). The provisionally infected larvae were treated with these drugs of 0.25, 0.50 and 1.00% concentration along with mulberry leaves once on alternate days till the onset of spinning. A control batch (without any treatment) was also kept for comparison.

RESULTS AND DISCUSSION

Observations on the *in vitro* studies of microsporidia spores after incubating in different concentrations of benzimidazole derivative were recorded and presented in Table 1. Among six drugs tested, mebendazole were found effective against microsporidian spores at all the concentrations. Rest of the drugs viz., metronidazole, albendazole, ornidazole and satinidizole were found effective only at 1%. However, tinidizole was not found effective against the microsporidian spores at any of the concentrations tested. The results of the *in vivo* observations showed that among tested benzimidazole derivatives, mebendazole (0.25 to 1.00%), albendazole (1.00%) were found effective in reduction of larval mortality to an extent of 100% (Table 2). However, the other drugs at concentrations ranging 0.25 to 1.00% reduced the mortality from 78.56 to 96.46%. At 1% concentration orinidazole reduced the larval mortality by 96.46%, followed by metronidazole 92.92% and satranidazole 92.92%.The percent of infected moths was significantly low in all treatments as compared to the inoculated control. It is recorded 59.38% in inoculated control was reduced to 22.44 to 30.04% in treated batches (Table 3).

Several therapeutic drugs have been identified as antimicrosporidia agents to control *Nosema. bombycis* infection in silkworm (Baig, 1994). In the present study benzimadizole derivatives were found effective in preventing larval mortality and also suppressing infection at moth stage as compared to control. A similar observation was made by Joythi et al. (2005) stated that when carbendazimin given 48 h post inoculation of *N. bombycis* reduced the infection but could not eliminated the infection at moth stage completely. Chandra et al. (1995) also reported 3 to 4% bavistin treatment twice a day reduced *N. bombycis* infection in silkworms. Analogues of benzimidazole and benlate, bavistin, derosal, Fumidil - B or fumagillin, methylthiophanate, ethyl thiophanate and anisomycin have been reported to

Table 2: Efficacy of benzimidazole derivatives in larval mortality.

Treatment	% mortality and disease reduction due to treatment			
	0.25%	0.50%	1.00%	Control
Metronidazole	1.33 ± 0.57 (85.74)	1.00 ± 0.00 (89.28)	0.66 ± 0.57 (92.92)	9.33 ± 0.57
Albendazole	1.33 ± 0.57 (85.74)	0.33 ± 0.57 (96.46)	0.00 ± 0.57 (100.0)	
Tinidizdazole	1.66 ± 0.57 (82.20)	1.66 ± 1.15 (82.20)	1.00 ± 1.00 (89.28)	
Ornidizole	2.00 ± 1.00 (78.56)	1.33 ± 0.57 (85.74)	0.33 ± 0.57 (96.46)	
Mebendazole	0.00 ± 1.00 (100.00)	0.00 ± 0.57 (100.0)	0.00 ± 0.57 (100.0)	
Satranidizole	1.66 ± 0.57 (82.20)	2.00 ± 1.00 (78.56)	0.66 ± 0.57 (92.92)	

Each value is mean±SD of three replications; values within parenthesis indicate % reduction in mortality.

Table 3. Efficacy of benzimidazole derivatives on suppression of infection at moth stage.

Treatment	% disease suppression at moth stage due to treatment			
	0.25%	0.50%	1.00%	Control
Metronidazole	25.18 ± 2.88 (59.59)	24.56 ± 0.66 (58.63)	23.40 ±1.54 (60.59)	59.38 ± 4.78
Albendazole	27.36 ± 1.06 (53.92)	22.76 ± 1.19 (61.67)	22.94 ± 0.51 (61.36)	
Tinidizdazole	25.56 ± 5.24 (56.95)	28.41 ± 1.98 (52.15)	27.44 ± 1.45 (53.78)	
Ornidizole	23.60 ± 3.55 (60.25)	30.04 ± 1.44 (49.41)	24.14 ± 1.81 (59.34)	
Mebendazole	22.44 ± 1.02 (62.20)	22.51 ± 2.63 (62.09)	23.79 ± 0.92 (59.93)	
Satranidizole	24.58 ± 2.52 (58.60)	24.05 ± 2.86 (59.49)	27.05 ± 1.61 (54.44)	

Each value is mean±SD of three replications; values within parenthesis indicate % reduction in infection.

be effective against different microsporidians. With observations of earlier studies, it is confirmed that benzimidazole derivatives are effective way to control microsporidiosis. Though the treatment controlled larval / moth mortality but the infection was again observed at moth stage. The present study is with agreement (Joythi et al., 2005; Brook et al., 1978) where they stated that chemical treatment arrested the further multiplication of microsporidians during feeding stage although they could not eliminate the disease completely and the development of some spore stages again started during non- feeding stage where feeding of chemicals/ drugs is impossible. Hence, for the complete elimination of microsporidiosis is only possible by feeding mulberry drug fortified leaves to silkworms continuously rather alternatively.

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