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ORIGINAL ARTICLE

Combinatorial Potentiality of *Aspergillus flavus* and *Cuscuta reflexa* against Mosquito Vectors

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ABSTRACT
The present study was conducted to determine the effectiveness of entomopathogenic fungus, Aspergillus flavus combined with petroleum ether extract of Cuscuta reflexa against Anopheles stephensi and Culex quinquefasciatus larvae. Larvicidal activity of both biopesticides was assessed separately and together against both the larvae. The combinatorial studies were done for different ratios, 1:1, 1:2 and 1:4. The results reveal that the ratio, 1:4 was the most effective than other tested combinations indicating highest synergistic activity. The LC_{50} values of ratio, 1:4 were 3.981, 3.319 and 2.392 mg/L and LC_{90} values with 23.426, 16.480 and 9.311 mg/L after 24, 48 and 72 hrs of post exposure respectively. It is an ideal eco-friendly approach for the control of mosquito vector. This study therefore, provides first report on the combined effect of larvicidal efficacy of the fungal mycotoxins of A. flavus and plant against both larvae.
KEYWORDS: *Anopheles stephensi, Aspergillus flavus, Culex quinquefasciatus, Cuscuta reflexa and Entomopathogenic fungus*

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INTRODUCTION

Mosquitoes, *An. stephensi* and *Cx. quinquefasciatus* are responsible for the transmission of several parasites that cause diseases like malaria and filariasis. In mosquito vector control, many efforts have been made in developing countries using insecticides. However, the continued use of this method has resulted in the development of mosquito resistance. For an alternative to chemical control, there is a resurgence of interest in the use of biopesticides. Therefore, biological control is an important component of the integrated vector control strategy. Among various biocontrol agents, plant extracts [1] and entomopathogenic fungi [2] belong to the most promising groups used for mosquito control. Biological control, including the use of enthomopathogenic fungi, as a part of an integrated pest management (IPM) strategy is expected to reduce the dependence on synthetic pesticides.

The synergists are considered straight forward tools for overcoming metabolic resistance and could be more effective than the individual components of the mixture. Thus, synergism has been preferred as an ideal strategy for resistance related problems, eco-friendliness and economical as it reduces the quantity of insecticide needed to kill the target population than the individual components of the mixture. A combination of entomopathogenic fungus with a plant-based insecticide may provide a more sustainable pest management strategy. Therefore, it is necessary to determine the compatibility of plants extract with entomopathogenic fungi [3].

The present research, demonstrated the compatibility of mycotoxins of *A. flavus* with petroleum ether extract (PEE) of *C. reflexa* for their simultaneous application to combat mosquito vectors.

MATERIALS AND METHODS

Mosquito Rearing: The mosquito vectors, *Cx. quinquefasciatus* and *An. stephensi* were reared in the laboratory, maintained continuously at 27±2º C and 70-80% relative humidity under a photoperiod of 14:10 h (light/dark) without exposure to pathogens or insecticides. The larvae were fed with powdered

brewer's yeast. Freshly molted larvae were continuously available for the mosquito larvicidal experiments.

Isolation of Fungus: *A. flavus* (MTCC No.- 1973) strain was obtained from the Institute of Microbial Technology, Chandigarh, India and stored at 4° C. Prior to testing on mosquito larvae it was cultured on Peptones (20g/L), dextrose (40g/L), potato dextrose agar (PDA: 20g/L) petriplates separately. The petriplates were placed in biological oxygen demand (BOD) incubator and held for 7 days. After 7 days, *Aspergillus* isolates were subcultured on Czepak solution agar media (sucrose 30g/L, agar 15g/L, $NaNO_3$ 2g/L, K_2HPO_4 1g/L, KCl 0.5g/L, $MgSO4.7H_2O$ 0.5g/L, and $FeSO_4.7H_2O$ 0.01g/L, at pH 7.3±0.2) to obtain pure cultures. *Aspergillus* species was determined morphologically under a microscope and isolates were stored at 4° C for further analysis [4].

Extraction of toxins: Isolates of *A. flavus* were cultured in 500 mL Erlenmeyer flasks containing 250 mL of sterile yeast extract sucrose (YES) liquid medium (20% sucrose and 5% yeast extract). The flasks were incubated separately for 7-10 days in the dark at 27-30° C without agitation. To lysed cells 25 mL of chloroform were added to recover mycelia and then agitated for 10 min on a rotator shaker. The flasks contents were filtered (Whatman no. 1) and the filtrate were used for toxin extraction. The filterate was transferred quantitatively to a separating funnel and extracted successively with 100 mL of chloroform to separate chloroform and aqueous layers. The procedure was repeated three times with lower transparent chloroform layer collected in a new flask. The chloroform was evaporated at 100 ° C by a vaccum rotatory evaporator to obtain the crude extract of each fungus [5]. The extracts were finally weighed and kept in refrigerator at 4° C until further use.

Phytoextract Preparation: The stems of *C. reflexa* were collected from the different locatilities of Agra. The stems were than washed in running tap water and dried in the shade. The shade dried stems were crushed mechanically and subjected to extraction with petroleum ether, hexane and methanol subsequently in a soxhlet apparatus (Borosil) for 72 hrs. Extracts were concentrated by removing the solvent by vaccum rotatory evaporator (Biocraft Scientific Industries, Agra, India). The extracts obtained as thick viscous paste were completely evaporated to dryness at room temperature and extracts are finally weighed and kept in refrigerator below 5°C until further use away from any chemical contact.

Bioassay of Fungal and Phytoextracts: For bioassay pure residues were dissolved in ethanol to get stock solutions. The crude extracts obtained from each solvent were dissolved independently in ethanol to obtain stock solutions of 50,000 mg/L individually. A range of working test concentrations was prepared for each extract by further diluting these stocks. The controls were exposed to the solvent, i.e., ethanol alone. The mortality data were recorded after 24, 48 and 72 hrs of exposure .All the experiments were conducted according to WHO standard procedure [6].

Combined biassay of Fungal and Phytoextract: For combinatorial studies, stock solution of *A. flavus* and the petroleum ether extract (PEE) of *C. reflexa* were prepared individually. Keeping *A. flavus* as the standard, its stock was mixed with the stock of PEE in ratios of 1:1, 1:2 and 1:4. A range of desired test concentrations for each mixed formulation ratio were prepared by further diluting the combination in water. The larvicidal efficacy of each formulation was observed as abovesaid.

Statistical Analysis: Mortality data obtained for the *A. flavus* and extracts of *C. reflexa* bioassays and their combinatorial studies were analyzed by Probit Analysis [7] to obtain LC_{50} and LC_{90}, standard error, regression equation and fiducial limits at 95% confidence limits. The co-toxicity coefficient [8] and synergistic factor [9] for the mixed formulation were also calculated after calculating LC_{50} and LC_{90} for each combination.

$$\text{Co-toxicity coefficient} = \frac{\text{Toxicity of insecticide (alone)}}{\text{Toxicity of insecticide with fungal extract}} \times 100$$

$$\text{Synergistic factor (SF)} = \frac{\text{Toxicity of insecticide (alone)}}{\text{Toxicity of insecticide with fungal extract}}$$

Value of SF > 1 indicates synergism and SF < 1 indicates antagonism

RESULTS

Bioassay of *A. flavus* and *C. reflexa*: Table 1 and 2 provides larval mortality after ethanolic extract, *A. flavus.* The LC_{50} value was 10.872, 8.153 and 7.049 mg/L after 24, 48 and 72 hrs of exposure. The LC_{90} value was 33.233, 247.286 and 19.550 mg/L after 24, 48 and 72 hrs of treatment, respectively against anopheline larvae. In case of culicine larvae, the LC_{50} value was 13.616, 14.347 and 10.027 mg/L after 24,

48 and 72 hrs of exposure. The LC_{90} value was 70.313, 67.474 and 45.691 mg/L after 24, 48 and 72 hrs of treatment, respectively.

The larvicidal potentiality of crude extracts of *C. reflexa* against *An. stephensi* was mentioned in table 1. The mortality data revealed that the petroleum ether extract (PEE) were the most effective followed by hexane and methanol extract. The PEE was most effective extract with LC_{50} 39.251, 33.180 and 20.032mg/L 24, 48 and 72 hrs of treatment. LC_{90} values were 292.935, 229.935 and 134.976 mg/L after 24, 48 and 72 hrs of treatment, respectively. The PEE
followed hexane with LC_{50} value of 87.085, 75.849 and 64.046mg/L after 24, 48 and 72 hrs of exposure and LC_{90} values of 285.926, 265.176 and 225.367 mg/L after 24, 48 and 72 hrs of treatment, respectively. The methanol extract possess least potency with LC_{50} value of 287.881, 266.595 and 223.492 mg/L after 24, 48 and 72 hrs of exposure and LC_{90} values of 770.601, 691.470 and 664.841mg/L after 24, 48 and 72 hrs of treatment, respectively.

Table 1 Larvicidal toxicity of extracts of *A. flavus* and *C. reflexa* individually against *An. stephensi*

Fungus and plant extracts	Exposure period (Hours)	Chi-square	Regression equation	LC_{50} ±SE (Fiducial limits) (mg/L)	LC_{90} ±SE (Fiducial limits) (mg/L)
A. flavus	24	0.187	2.642x+7.978	10.872±2.019 (14.830-6.913)	33.233±14.572 (61.784-4.662)
	48	0.826	2.443x+0.331	8.153±1.857 (11.793-4.513)	27.286±11.306 (49.446-5.125)
	72	1.411	7.049x+1.536	7.049±1.536 (10.060-4.038)	19.550±5.565 (30.457-8.642)
Petroleum ether	24	2.960	1.469x+1.191	39.251±11.953 (62.679-15.823)	292.771±143.928 (574.871-10.671)
	48	2.419	1.524x+1.157	33.180±10.353 (53.473-12.888)	229.935±102.579 (430.991-28.879)
	72	1.471	1.547x+1.439	20.032±6.907 (33.570-6.495)	134.976 ± 52.376 (237.634-32.318)
Hexane	24	1.957	2.482x-2.298	87.085±24.153 (134.426-39.744)	285.926±78.438 (439.665-132.187)
	48	1.677	2.358x-1.790	75.849±24.895 (124.643-27.054)	265.176±73.849 (409.922-120.431)
	72	1.098	2.345x-1.583	64.046±24.370 (111.812-16.280)	225.367±58.751 (340.518-110.216)
Methanol	24	1.072	2.997x-5.367	287.881±40.188 (366.651-209.112)	770.601±213.792 (1189.633-351.569)
	48	1.587	3.096x-5.607	266.595±36.734 (338.594-194.596)	691.470±171.875 (1028.346-354.594)
	72	1.036	2.707x-4.066	223.492±36.531 (295.093-151.891)	664.841±182.703 (1022.939-306.743)

Table 2 reveals the larval mortality data of crude extracts of *C. reflexa* against *Cx. quinquefasciatus*. The PEE was most effective extract with LC_{50} 48.625, 31.869 and 21.667 mg/L 24, 48 and 72 hrs of treatment. LC_{90} values were 266.272, 175.041 and 156.014 mg/L after 24, 48 and 72 hrs of treatment, respectively. The PEE followed hexane with LC_{50} value of 94.995, 63.412 and 47.275 mg/L after 24, 48 and 72 hrs of exposure and LC_{90} values of 260.085, 201.819 and 181.557 mg/L after 24, 48 and 72 hrs of treatment, respectively. The methanol extract possess least potency with LC_{50} value of 320.126, 285.519 and 241.274 mg/L after 24, 48 and 72 hrs of exposure and LC_{90} values of 814.202, 726.448 and 601.349 mg/L after 24, 48 and 72 hrs of treatment, respectively.

Table 2 Larvicidal toxicity of extracts of *A. flavus* and *C. reflexa* individually against *Cx. quinquefasciatus*

Fungus and plant extracts	Exposure period (Hours)	Chi-square	Regression equation	LC_{50} ±SE (Fiducial limits) (mg/L)	LC_{90} ±SE (Fiducial limits) (mg/L)
A. flavus	24	7.864	1.797x+1.164	13.616±2.355 (18.233-9.000)	70.313±29.017 (127.187-13.439)
	48	8.097	1.906x+0.889	14.347±2.399 (19.049-9.644)	67.474±26.101 (118.633-16.316)
	72	10.855	1.946x+1.106	10.027±1.515 (12.996-7.058)	45.691±14.587 (74.282-17.099)
Petroleum ether	24	3.655	1.735x+0.337	48.625±12.680 (73.478-23.771)	266.272±111.698 (485.201-47.343)
	48	2.487	1.732x+0.663	31.869±9.126 (49.755-13.982)	175.041±64.716 (301.885-48.196)

	72	1.1580	1.495x+1.508	21.667±7.486 (36.341-6.994)	156.014±64.051 (281.555-30.474)
Hexane	24	2.205	2.930x-3.724	94.995 ±21.040 (136.233-53.757)	260.085±51.686 (361.390-158.780)
	48	5.964	2.549x-2.143	63.412 ±22.089 (106.706-20.118)	201.819±44.091 (288.237-115.400)
	72	5.998	2.193x-0.866	47.275±24.009 (94.334-0.215)	181.557±44.458 (268.696-94.419)
Methanol	24	2.324	3.161x-6.081	320.126 ±42.170 (402.778-237.47)	814.202±224.876 (1254.96-373.443)
	48	2.064	3.160x-5.920	285.519 ±38.428 (360.839-210.10)	726.448±185.134 (1089.31-363.486)
	72	2.202	3.231x-5.930	241.274±33.241 (306.426-176.122)	601.349±132.687 (861.416-341.281)

Combinatorial bioassay

The combinatorial bioassay of *A. flavus* and PEE of *C. reflexa* against anopheline larvae were depicted in table 3 and Fig 1. Synergistic factor has been worked out and the highest synergism was found to be in 1:4 as compared to 1:2 and 1:1. The ratio 1:1 had LC_{50} value 7.910, 6.368 and 4.317 mg/L after 24, 48 and 72 hrs of exposure, respectively. The LC_{90} value was 17.452, 15.525 and 15.627 mg/L after 24, 48 and 72 hrs of exposure, respectively. The co-toxicity coefficient 137.446, 128.031 and 163.285 and synergistic factor 1.374, 1.280 and 1.633 indicates synergism in LC_{50} values after 24, 48 and 72 hours. The co-toxicity coefficient 190.425, 175.755 and 125.104 and the synergistic factor 1.904, 1.757 and 1.251 indicates synergism in LC_{90} values after 24, 48 and 72 hours, accordingly. The LC_{50} value for ratio 1:2 was 5.689, 5.169 and 4.759 mg/L after 24, 48 and 72 hrs of treatment period. The LC_{90} value was 10.519, 9.368 and 8.088 mg/L after 24, 48 and 72 hrs of exposure, respectively. The co-toxicity coefficient 191.105, 157.729 and 148.119 and the synergistic factor 1.911, 1.577 and 1.481 indicates synergism in LC_{50} values after 24, 48 and 72 hours. The co-toxicity coefficient 315.933, 291.268 and 241.716 and the synergistic factor 3.159, 2.913 and 2.417 indicates synergism in LC_{90} values after 24, 48 and 72 hours, respectively. For ratio 1:4 the LC_{50} value was 3.981, 3.319 and 2.392 mg/L after 24, 48 and 72 hrs of exposure, respectively. The LC_{90} value was 23.426, 16.480 and 9.311 mg/L after 24, 48 and 72 hrs of exposure, respectively. The co-toxicity coefficient 273.097, 245.646 and 294.691 and synergistic factor 2.731, 2.456 and 2.947 indicates synergism in LC_{50} values after 24, 48 and 72 hours. The co-toxicity coefficient 141.864, 165.570 and 209.967 and the synergistic factor 1.419, 1.656 and 2.099 indicates synergism in LC_{90} values after 24, 48 and 72 hours of exposure period.

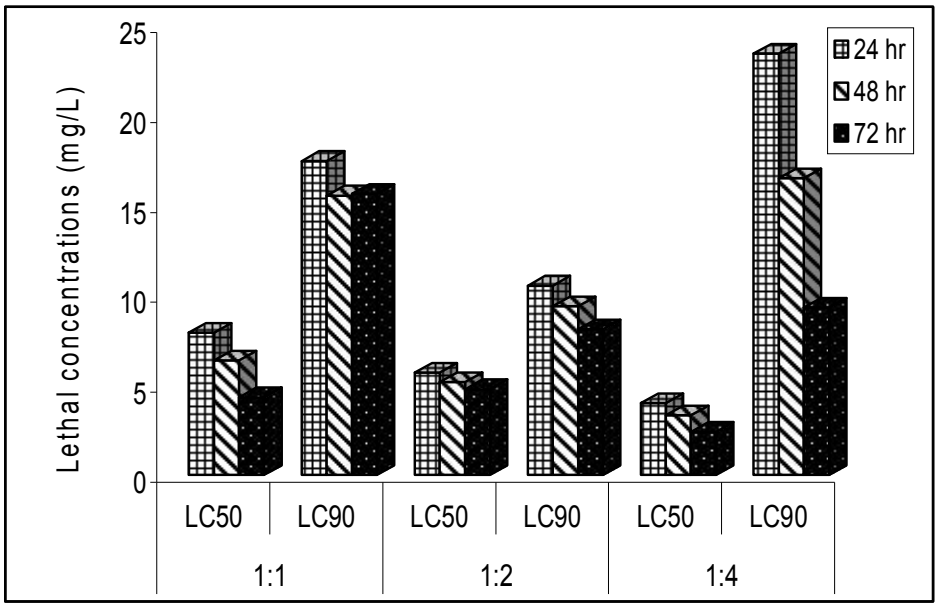

Fig. 1 Comparative larvicidal potentiality of *A.* flavus and PEE of *C. reflexa* against *An. stephensi*

Table 3 Combinatorial toxicity of different combination ratios of *A. flavus* with petroleum ether extract (Pee) of *C. reflexa* against anopheline larvae

Ratio	Exposure period (Hours)	Chi-square	Regression equation	LC_{50} ±SE (Fiducial limits) (mg/L)	SF	CTC	Type of action	LC_{90} ±SE (Fiducial limits) (mg/L)	SF	CTC	Type of action
1:1	24	1.682	3.729x-2.079	7.910±0.969 (9.811-6.010)	1.374	137.446	S	17.452±3.854 (25.005-9.899)	1.904	190.425	S
	48	1.235	3.311x-0.973	6.368±0.925 (8.180-4.555)	1.280	128.031	S	15.525±3.550 (22.482-8.567)	1.757	175.755	S
	72	1.273	2.294x+1.249	4.317±1.191 (6.652-1.983)	1.633	163.285	S	15.627±4.652 (24.745-6.509)	1.251	125.104	S
1:2	24	1.222	4.801x-3.427	5.689±0.641 (6.945-4.434)	1.911	191.105	S	10.519±1.749 (13.947-7.091)	3.159	315.933	S
	48	0.985	4.963x-3.504	5.169±0.626 (6.396-3.942)	1.577	157.729	S	9.368±1.433 (12.177-6.559)	2.913	291.268	S
	72	1.054	5.565x-4.336	4.759±0.568 (5.873-3.645)	1.481	148.119	S	8.088±1.077 (10.198-5.977)	2.417	241.716	S
1:4	24	0.544	1.665x+2.336	3.981±0.918 (5.781-2.181)	2.731	273.097	S	23.426±11.541 (46.047-0.806)	1.419	141.864	S
	48	0.401	1.841x+2.199	3.319±0.715 (4.721-1.917)	2.456	245.646	S	16.480±7.165 (30.522-2.437)	1.656	165.570	S
	72	2.012	2.172x+2.005	2.392±0.487 (3.346-1.439)	2.947	294.691	S	9.311±3.071 (15.330-3.291)	2.099	209.967	S

CTC, Co-toxicity coefficient; SF, Synergistic Factor

Table 4 reveals the combinatorial bioassay of *A. flavus* and PEE of *C. reflexa* against culicine larvae. The ratio 1:1 had LC_{50} value 8.712, 6.671 and 5.755 mg/L after 24, 48 and 72 hrs of exposure, respectively. The LC_{90} value was 15.763, 12.993 and 11.781 mg/L after 24, 48 and 72 hrs of exposure, respectively. The LC_{50} value for ratio 1:2 was 6.030, 5.753 and 5.409 mg/L after 24, 48 and 72 hrs of treatment period. The LC_{90} value was 9.555, 10.047 and 10.344 mg/L after 24, 48 and 72 hrs of exposure, respectively. The ratio 1:4 have the LC_{50} value 5.444, 4.094 and 4.482 mg/L after 24, 48 and 72 hrs of exposure, respectively. The LC_{90} value was 24.458, 20.570 and 20.764 mg/L after 24, 48 and 72 hrs of exposure period (Fig 2).

Table 4 Combinatorial toxicity of different combination ratios of *A. flavus* extract with petroleum ether extract (Pee) of *C. reflexa* against culicine larvae

Ratio	Exposure period (Hours)	Chi-square	Regression equation	LC_{50} ±SE (Fiducial limits) (mg/L)	SF	CTC	Type of action	LC_{90} ±SE (Fiducial limits) (mg/L)	SF	CTC	Type of action
1:1	24	5.537	4.977x-4.656	8.712±0.883 (10.443-6.981)	1.563	156.29	S	15.763±2.957 (21.558-9.967)	4.461	446.063	S
	48	8.656	4.427x-3.076	6.671±0.740 (8.123-5.220)	2.151	215.065	S	12.993±2.498 (17.889-8.096)	5.193	519.310	S
	72	5.701	4.119x-2.250	5.755±0.741 (7.207-4.304)	1.742	174.231	S	11.781±2.235 (16.162-7.401)	3.878	387.836	S
1:2	24	40.808	6.411x-6.415	6.030±0.516 (7.043-5.018)	2.258	225.804	S	9.555±1.204 (11.916-7.194)	7.359	735.876	S
	48	28.040	5.292x-4.314	5.753±0.567 (6.865-4.641)	2.494	249.383	S	10.047±1.467 (12.923-7.172)	6.716	671.583	S
	72	20.179	4.551x-2.887	5.409±0.604 (6.592-4.225)	1.854	185.376	S	10.344±1.679 (13.635-7.054)	4.417	441.715	S
1:4	24	1.874	1.964x+1.590	5.444±1.133 (7.666-3.222)	2.501	250.110	S	24.458±10.893 (45.808-3.107)	2.874	287.485	S
	48	0.452	1.828x+2.053	4.094±0.873 (5.805-2.383)	3.504	350.440	S	20.570±9.326 (38.848-2.291)	3.280	328.021	S
	72	0.402	1.925x+1.821	4.482±0.924 (6.293-2.671)	2.237	223.717	S	20.764±9.072 (38.545-2.983)	2.200	220.049	S

CTC, Co-toxicity coefficient; SF, Synergistic Factor

The co-toxicity coefficient for the 1:1 were 156.29, 215.065 and 174.231 with synergistic factor 1.563, 2.151 and 1.742 at 24, 48 and 72 hrs respectively for the LC_{50} which indicates synergism and with the LC_{90} co-toxicity coefficient was 446.063, 519.310 and 387.836 with synergistic factor 4.461, 5.193 and 3.878 showing synergism at 24, 48 and 72 hrs respectively. For ratio 1:2, the co-toxicity coefficient values at LC_{50} were 225.804, 249.383 and 185.376 with synergistic factor 2.258, 2.494 and 1.854 shows synergism after 24, 48 and 72 hrs of exposure respectively. The co-toxicity coefficient values at LC_{90} were 735.876, 671.583 and 441.715 with synergistic factor 7.359, 6.716 and 4.417 which shows synergism after 24, 48 and 72 of exposure. For ratio 1:4, the co-toxicity coefficient was 250.110, 350.440 and 223.717 with synergistic factor 2.501, 3.504 and 2.237 at LC_{50} and shows synergism after 24, 48 and 72 hrs of treatment. The LC_{90} had the co-toxicity coefficient 287.485, 328.021 and 220.049 with synergistic factor 2.874, 3.280 and 2.200 which shows synergism after 24, 48 and 72 hrs of exposure accordingly.

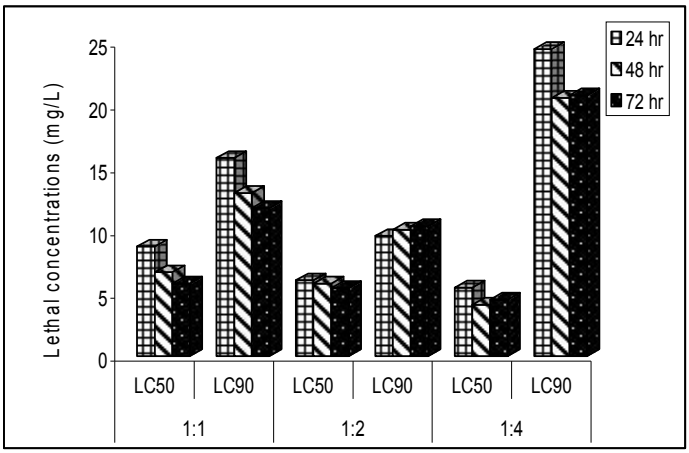

Fig. 2 Comparative larvicidal potentiality of *A. flavus* and PEE of *C. reflexa against Cx. quinquefasciatus*

DISCUSSION

The present study reveals that the combinatorial bioassay of *A. flavus* and PEE of *C. reflexa* was found more effective to both the larvae, anopheline and culicine larvae with synergistic action at each ratio. Synergism between *A. flavus* and PEE of *C. reflexa* could allow for reductions in amount of biopesticide concentrations used in mosquito control, thereby restriction on insecticide resistance as well as other negative environmental impacts. Synergistic effects between entomopathogenic fungi and insecticides have been examined by many researchers which influence the synergism. Hiromori and Nishigaki [10] reported synergistic effects of an entomopathogenic fungus, *Metarhizium anisopliae* and insecticides against larvae, *Anomala. cuprea.* Boucias et al. [11] showed that the synergistic effects of *Beauveria bassiana* and imidacroprid on the termite *Reticulitermes flavipes* caused an altered behavior that could be disrupted with sublethal dosages of imidacroprid. Quintela and McCoy [12] also demonstrated that the synergistic effects among *B. bassiana*, *M. anisopliae* and imidacroprid to *Diaprepes abbreviatus*, resulted in an insecticidal effect on the behavior of *D. abbreviatus.* They believed that a sublethal dose of imidacroprid inhibited the behavior of *D. abbreviatus*, making it difficult to remove the conidia from the cuticle surface. Synergistic effect of some entomopathogenic fungi and synthetic pesticides, were reported against two spotted spider mite, *Tetranychus urticae* [13].

The application of fungal mycotoxins combined with botanicals is an important tactics to be utilized in mosquito control. During current study in each combined treatment a higher mortality was observed as compared to the fungus or botanical alone. Our work is favorably supported by the findings of various researchers. Kumar et al. [14] determine the effectiveness of seaweed (*Sargassum wightii*) extract combined with *Bacillus thuringiensis var. israelensis* for the control of *Anopheles sundaicus* and found II instar was the most susceptible.

Hemocytes of larvae were significantly affected by a combined action of *A.flavus* and insecticides. The results indicated that the synergism might be caused by the inhibitor of the larvae cellular immune system [15]. Furthermore, phenoloxidase (PO) activity of mosquito larvae was inhibited by the mixed application of fungus and insecticides. Melanization depending on the activation of the PO cascade is one of the major defenses of the humoral reaction against non-self [15]. Results also showed that the synergism might be caused by the inhibition of the larval humoral defense system.

Hiromori and Nishigaki [16] founded synergism between *Metarhizium anisopliae* and fenitrothion or teflubenzuron against scarab beetle larvae; they attributed the synergy to weakening of the immune system by insecticidal stress and facilitating infection of *M. anisopliae* to the larvae. Hornbostel *et al.* [17] investigated that combination of permethrin with *M. anisopliae* was highly effective in *Ixodes. scapularis* control options by inducing the highest mortality (approximately 90%). Santos *et al.* [18] showed that when imidacloprid mixed with *B. bassiana* CG24, show higher mortality of insects than single.

In this context, this present study provides the compatibility between *A. flavus* extract and PEE of *C. reflexa* on mosquito larvae. The tested individual botanicals and fungi have also been used in mosquito management. However, they are comparatively less active than their combination. The larval mortality was caused by inhibition of the immune reaction.

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