

Eco-friendly management of *Sciara orientalis* in *Agaricus bisporus* with agniastra, seed kernel extracts of *Melia azedarach* and different colored sticky traps and light traps

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ABSTRACT

White button mushroom is very important indoor crop which has been eaten and appreciated for its taste, economic values, flavor and medicinal properties for years. It is a very important medicinal and nutritional species which is used to recycle agro wastes including waste paper, wheat straw, waste tea leaves, oat straw and some water plants. Other than that they are valued from the nutritional point of view. Most common method for their control has been chemical control but due to environment safety and food safety issues, alternative methods should be used. Mushroom cultivation is greatly affected by a range of insect- pests which can cause serious crop loss. Out of all the pests, sciarid flies are key pests of button mushroom. Therefore, seed kernel extracts (aqueous, ethanolic and diethyl ether) of *Melia azedarach* and *agniastra* (2.0 %) were evaluated for their effect on mycelial growth of *Agaricus bisporus*, larval mortality and repellency of *Sciara orientalis*. *Agniastra* (2 %) and aqueous seed kernel extracts (10 %) showed 0.00 per cent mushroom mycelium inhibition and maximum larval mortality. LC50 and LC90 values were also calculated for seed kernel extracts by using probit analysis and it was observed that with increase in exposure period from 24 to 48 h, a significant decrease in concentration occurs. Different colored traps were also tested for their efficacy in catching flies, out of which yellow trap without bulb and white trap with bulb were found most efficient. Among seed kernel extracts, diethyl ether extracts showed maximum per cent repellency.

INTRODUCTION

White button mushroom is very important indoor crop which has been eaten and appreciated for its taste, economic values, flavor and medicinal properties for years. It is a very important medicinal and nutritional species which is used to recycle agro wastes including waste paper, wheat straw, waste tea leaves, oat straw and some water plants [1]. Other than that they are valued from the nutritional point of view [2]. Mushroom is an excellent source of iron for anemic patients [3]. Nanoparticles synthesized from *A. bisporus* are used to treat cancer, viral, bacterial and fungal diseases [4]. Mushroom cultivation is greatly affected by a range of insect-pests which can cause serious crop loss. Out of all the pests, sciarid flies are key pests of button mushroom. These flies are commonly known as mushroom flies, fungus gnat, nuisance flies and dark colored flies belonging to family Sciaridae [5]. Maggots of this fly causes most of the damage by tunneling the stipe to reach the sporocarp and adult causes brown patches on the fruiting bodies, which decreases its market value. These flies also vector some diseases, mites and nematodes [6]. In total 66.70 per cent crop losses were caused by this fly in Punjab, which necessitate the management of this fly [7].

Most common method for their control has been chemical control but due to environment safety and food safety issues, alternative methods should be used. Several management tactics have been worked out under Indian conditions and use of plant products were more successful in maximizing the yield and minimizing pest damage to mushrooms [8]. Plant-based products can be recommended as a substitute for synthetic chemicals in the commercial production of edible mushrooms. Various formulations of these plant-derived products have been found effective through different modes of action against insects and mites i.e. growth regulators, anti-feedants, pesticides and repellents [9]. In this endeavor to search for non-chemical control method, sticky trap using different colored lights can also be used [10].

Materials and methods

2.1 Maintenance of culture in laboratory

The compost samples were collected from mushroom bags infested with flies. These samples having egg, larval and pupal stages were kept in the plastic cages and 500g fresh spawned mushroom compost was provided in the cage. The culture of sciarid fly was maintained by daily sprinkling of water and by keeping the cages in dark places. This culture of the flies was then used for further studies.

2.2 Collection of seed kernels of *M. azedarach*

Seed kernels of *M. azedarach* were collected from the surroundings of Hamirpur (H.P.). These seed kernel were shade dried and a coarse powder was made by pounding it with a wooden stick. This powder made was stored in cool and dry place in the room and was used for further experiments.

2.3 Preparation of aqueous, ethanolic and diethyl ether seed kernel extracts of *M. azedarach*

With the help of electric grinder, shade dried leaves were powdered. 40.0 g of powdered sample was taken and soaked in 200 ml distilled water/ethanol/diethyl ether overnight in a glass beaker of 500 ml capacity at ambient room temperature. The solvents were evaporated and extract was further dried. The dried extract was again soaked in 200 ml distilled water and was filtered 3-4 times with a fine muslin cloth. This stock solution was designated as 20.0 per cent seed kernel extract and was stored in refrigerator for further preparation of doses.

2.4 Preparation of doses

Twenty per cent seed kernel extract of dharek (*M. azedarach*) prepared by using water, ethanol and diethyl ether solvents was used for further preparation of doses. Different concentrations were prepared from the 20.0 per cent stock solutions of aqueous extracts (5.0, 7.0 and 10.0 %), ethanolic extracts (7.0, 9.0 and 10.0 %) and diethyl ether extracts (8.0, 9.0 and 10.0 %). Each concentration of seed kernel extract prepared was filtered through Whatman No.1 filter paper. By using poison food technique, the filtered extracts after sterilization were poured in compost agar media to grow mycelium of *A. bisporus*.

2.5 Preparation of compost extract agar medium

The flies were reared on the artificial media prepared by using compost agar

- i. The compost agar medium was prepared by boiling 250 g of compost in 1000ml of distilled water for one hour and was left for 24 h.
- ii. It was then filtered with muslin cloth and the volume was again made 1000ml by adding distilled water to it.
- iii. 20.0 g of agar-agar was added with continuous stirring till all the constituents were thoroughly mixed and final volume was made up to 1000 ml.
- iv. pH of the medium was adjusted to seven.
- v. The medium so prepared was poured in conical flasks and these were plugged tightly with non-absorbent cotton.
- vi. The medium was sterilized for twenty minutes at 15 psi pressure and 121 ° C temperature in an autoclave
- vii. The medium thus prepared was poured in sterilized Petri plates under aseptic conditions and allowed to solidify.

2.6 Preparation of Agniastra

By using mortar and pestle, garlic (50 g), green chilies (50 g), tobacco (50 g) and neem leaves (500 g) were crushed and grinded to make a fine paste which was mixed with 1500 ml cow urine. This solution was boiled four times and left for 48 h. After that it was filtered with a fine muslin cloth and 2.0 liter of solution was made by adding water. This solution was designated as 2.0 per cent agniastra.

2.7 Effect of agniastra and seed kernel extracts of *M. azedarach* on mycelial growth of button mushroom and larval mortality of *S. orientalis*

The effect of agniastra (2.0 %) and seed kernel extracts on per cent mycelial inhibition and per cent larval mortality was evaluated.

$$\text{Per cent mycelial inhibition} = \frac{C-T}{C} \times 100$$

Where, C= Colony diameter in control and T = Colony diameter in treatment

$$\text{Per cent larval mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae}} \times 100$$

After calculating the per cent mortality at different concentration, LC50 and LC90 values were also calculated with the help of probit analysis (Finney, 1971).

2.8 To study the repellent property of *M. azedarach*

Repellent effect of aqueous, ethanol and diethyl ether extracts of *M. azedarach* was tested against major mushroom fly *S. orientalis*. 10.0 per cent concentration of each seed kernel extracts was prepared and 100 ml capacity beakers filled with fresh spawned compost were treated with these formulations. These beakers along with one untreated beaker were kept in a cage and forty adult flies were released into this cage and number of adults landing on each treated as well as untreated compost was counted after 24 h of release.

$$\text{Per cent repellency} = \frac{C-T}{C} \times 100$$

Where, C= Average number of flies landing on control

T= Average number of flies landing on treated compost

2.9 Evaluation of different colored sticky traps against mushroom flies

Double sided different colored sticky traps (10×20 cm) viz. yellow, blue, green, red and white with bulbs (0.5 Watt) and without bulbs were placed inside the rooms. The traps were divided into squares and coated with mustard oil. All the five bulbs were installed at a constant distance of 1.5 to 2.0 meter to attract the adult flies and same colored trap of size 10×20 cm was also hung below each bulb. Traps were fixed at one position throughout the period of seven days. The bulb was switched on in the evening h after 5pm. Adult flies of *S. orientalis* were introduced into that room. Color of the trap preferred by the pest was noted down and number of insects stuck on each trap were counted on 7th day of release.

Results and discussion

Three sets of experiments were laid under this head. Evaluation of aqueous, ethanolic and diethyl ether solvents of seed kernel extracts of *Melia azedarach* and *agniastra* (2.0 %) was done at different concentrations viz., aqueous extracts (5.0 %, 7.0 % and 10.0 %), ethanol extracts (7.0 %, 9.0 % and 10.0 %) and diethyl ether extracts (8.0 %, 9.0 % and 10.0 %) to test their effect on mycelial growth of *A. bisporus* and on *S. orientalis*.

In first set, seed kernel extracts along with *agniastra* were tested for their effect on mycelial growth of *A. bisporus*, in the second set, same treatments were evaluated against *S. orientalis* to test the per cent larval mortality and in third set, the same treatments were screened for their effect on per cent repellent rate.

3.1 Effect on mycelial growth of *A. bisporus*

Agniastra, aqueous, ethanol and diethyl ether seed kernel extracts of *M. azedarach* were prepared as mentioned under 2.3 and were allowed to grow alongside the untreated control to check the effects of different extracts on growth of *A. bisporus*.



Plate 1 Mycelial growth shown by *agniastra* (2.0 %)



Plate 2 Mycelial Growth shown by aqueous seed kernel extracts of *M. azedarach*



Plate 3 Mycelial inhibition shown by ethanol seed kernel extracts of *M. azedarach*



Plate 4 Mycelial inhibition shown by diethyl ether seed kernel extracts of *M. azedarach*

Of all the seed kernel extracts of *M. azedarach*, mycelium inhibition was found to be maximum in ethanol extracts followed by diethyl ether extracts whereas aqueous seed kernel extracts and agniastra showed 0.00 per cent mycelium inhibition. Trends were similar in Table 1 that mycelium of *A. bisporus* was completely inhibited by 7.0 per cent, 9.0 per cent and 10.0 per cent concentration of ethanol seed kernel extracts of *M. azedarach*.

Table 1. Effect of agniastra and seed kernel extracts of *M. azedarach* on per cent mycelial inhibition of *A. bisporus*

Treatments	Per cent mycelial inhibition of <i>A. bisporus</i>
Aqueous seed kernel extract of <i>M. azedarach</i> (5.0 %)	0.00 (1.00)
Aqueous seed kernel extract of <i>M. azedarach</i> (7.0 %)	0.00 (1.00)
Aqueous seed kernel extract of <i>M. azedarach</i> (10.0 %)	0.00 (1.00)
Ethanol seed kernel extract of <i>M. azedarach</i> (7.0 %)	100.00 (10.05)
Ethanol seed kernel extract of <i>M. azedarach</i> (9.0 %)	100.00 (10.05)
Ethanol seed kernel extract of <i>M. azedarach</i> (10.0%)	100.00 (10.05)
Diethyl ether seed kernel extract of <i>M. azedarach</i> (8.0 %)	10.24 (3.35)
Diethyl ether seed kernel extract of <i>M. azedarach</i> (9.0 %)	14.04

	(3.88)
Diethyl ether seed kernel extract of <i>M. azedarach</i> (10.0 %)	16.43 (4.17)
Agniastra	0.000 (1.00)
Control	0.000 (1.00)
C.D.	0.10
SE(m)	0.03
SE(d)	0.05
C.V.	1.36

Figures in the parentheses are square root transformed values

0.00 per cent mycelial inhibition was recorded in case of aqueous seed kernel extracts at 5.0, 7.0 and 10.0 per cent concentrations and agniastra at 2.0 per cent along with control. 10.24 per cent mycelial inhibition was attained in diethyl ether seed kernel extract at 8.0 per cent concentration and with increase in concentration per cent mycelial inhibition also increased from 9.0 per cent (14.04 %) to 10.0 per cent (16.43 %) concentration.

3.2 Effect on per cent larval mortality of *S. orientalis*

Twenty, 2nd and 3rd instar larvae of *S. orientalis* were selected in each replication and were released into fully grown Petri plates of *A. bisporus* with different concentrations of aqueous extracts (5.0 %, 7.0 % and 10.0 %) and diethyl ether extracts (8.0 %, 9.0 % and 10.0 %). Per cent larval mortality of each concentration at an exposure periods of 24 and 48 h, along with uninoculated and untreated control was tested. As it is clear from 3.1 that ethanol seed kernel extracts of *M. azedarach* showed 100.00 per cent inhibition to *A. bisporus* at all three concentrations, so were not evaluated for larval mortality of *S. orientalis*.

Bhat et al. tested effect of different bacteria on growth of *A. bisporus* and found that 100.00 per cent mycelial inhibition of *A. bisporus* was shown by *B. thuringiensis* at 60.00 per cent concentration followed by 73.28 per cent mycelial inhibition at 40.00 per cent concentration.

3.2.1 Efficacy of agniastra and seed kernel extracts of *M. azedarach* and agniastra on per cent larval mortality

It is very evident from the Table 2 that a significant increase in mortality was recorded with the increase in exposure period as well as concentration.

Table 2. Effect of seed kernel extracts of *M. azedarach* and agniastra on larval mortality of *S. orientalis* at different exposure periods

Treatments	Conc. (%)	Per cent mortality at exposure period (h)		Mean
		24	48	
Aqueous seed kernel extract of <i>M. azedarach</i>	5.0	36.67 (37.19)	45.00 (42.10)	40.83 (39.64)
Aqueous seed kernel extract of <i>M.</i>	7.0	55.00	63.33	59.17

<i>azedarach</i>		(47.86)	(52.72)	(50.29)
Aqueous seed kernel extract of <i>M. azedarach</i>	10.0	81.67 (64.78)	90.00 (71.92)	85.84 (68.35)
Diethyl ether seed kernel extract of <i>M. azedarach</i>	8.0	21.67 (27.70)	30.00 (33.15)	25.83 (30.42)
Diethyl ether seed kernel extract of <i>M. azedarach</i>	9.0	33.33 (35.23)	43.33 (41.15)	38.33 (38.19)
Diethyl ether seed kernel extract of <i>M. azedarach</i>	10.0	51.67 (45.94)	58.33 (49.78)	55.00 (47.86)
Agniastra	2.0	53.34 (46.89)	81.67 (64.67)	67.50 (55.78)
Standard (Nuvan)	0.1	86.67 (68.64)	93.33 (75.21)	90.00 (71.92)
Untreated control		0	0	
Mean		46.67 (41.58)	56.11 (47.86)	
CD _{0.05}				
	Treatment (T)	3.27		
	Exposure Period (P)	1.54		
	T × P	4.62		

Figures in the parentheses are angular transformed values

Highest mean per cent mortality of 85.84 per cent was attained in aqueous seed kernel extract of *M. azedarach* at 10.0 per cent concentration, followed by agniastra with 67.50 per cent mean larval mortality attained at 2.0 per cent concentration which was significantly lower than the standard check nuvan (0.1 %) with 90.00 per cent mortality. Mean per cent larval mortality of 25.83 achieved at 8.0 per cent concentration of diethyl ether seed kernel extract was lowest followed by 38.33 per cent mortality attained at 9.0 per cent concentration of the same extract.

The per cent mortality enhanced from 24 h of exposure period to 48 h of exposure period in all the treatments. Per cent mortality at 10.0 per cent concentration in aqueous seed kernel extract enhanced from 81.67 per cent at 24 h exposure period to 90.00 per cent at 48 h. In case agniastra, the enhancement from 53.34 per cent to 81.67 per cent larval mortality at 2.0 per cent concentration and similar exposure periods was recorded. Minimum per cent larval mortality was seen in control. Overall rate of mortality was high in case of nuvan (90.00 %) at 0.1 per cent concentration followed by aqueous seed extracts of *M. azedarach* and agniastra. Out of all the treatments, highest per cent larval mortality of 90.00 was attained by aqueous seed extract at 10.0 per cent concentration after 48 h of exposure period followed by 81.67 per cent mortality at same concentration of the same extract after an interval of 24 h and agniastra (2.0 %) with 81.67 per cent mortality after an exposure period of 48 h.

Nathan et al. (2006) tested seed kernel and leaf extracts of *M. azedarach* to check the larval mortality of *Anopheles stephensi* and results showed that larval mortality was more in seed extracts as compared to leaves. Chiffelle et al. (2019) reported the highest mortality of 63.00 per cent was in case of aqueous seed extract of *M. azedarach* at 4.4 per cent concentration followed by 52.00 per cent mortality at 3.0 per cent concentration against *Xanthogaleruca luteola* larvae. Management of sciarid and phorid fly on oyster mushroom by using different plant extracts at different concentration was done by Joshi (2009). In his studies he reported that aqueous water extracts of *M. azedarach* at 10.0 per cent concentration was statistically at par in controlling the mushroom sciarid fly as well as reducing the per cent infestation on fruiting bodies.

3.2.3 Estimation of LC₅₀ and LC₉₀

LC₅₀ and LC₉₀ –for different –seed kernel extracts was also calculated after calculation of larval mortality. It was calculated by using probit analysis at 24 h and 48 h of exposure period. The results presented in Table 3 revealed that LC₅₀ and LC₉₀ –value against *S. orientalis* after 24 hours was found to be 6.16 and 12.58 per cent and at 48 hours, it was 5.49 and 10.23 per cent in case of aqueous seed kernel extracts of *M. azedarach*. LC₅₀ and LC₉₀ values calculated for diethyl ether seed kernel extracts of *M. azedarach* was 9.96 and 14.12 after 24 hours and 9.33 and 13.80 after 48 hours of exposure period. A significant decrease in the values of LC₅₀ and LC₉₀ with increase in exposure period from 24 to 48 h is evident from Table 3.

Chiffelle et al. tested six concentrations of aqueous extracts of *M. azedarach* against the third instar larvae to check the mortality as well as LC₅₀ concentration at 24 hours interval and LC₅₀ of 1.49 per cent was found on eight day of exposure with aqueous seed extracts [12].

Table 3. Estimation of LC₅₀ and LC₉₀ of seed kernel extracts of *M. azedarach* for *S. orientalis*

Treatments	24 h		48 h	
	LC ₅₀ (%)	LC ₉₀ (%)	LC ₅₀ (%)	LC ₉₀ (%)
Aqueous seed kernel extracts of <i>M. azedarach</i>	6.16	12.58	5.49	10.23
Diethyl ether seed kernel extracts of <i>M. azedarach</i>	9.96	14.12	9.33	13.80

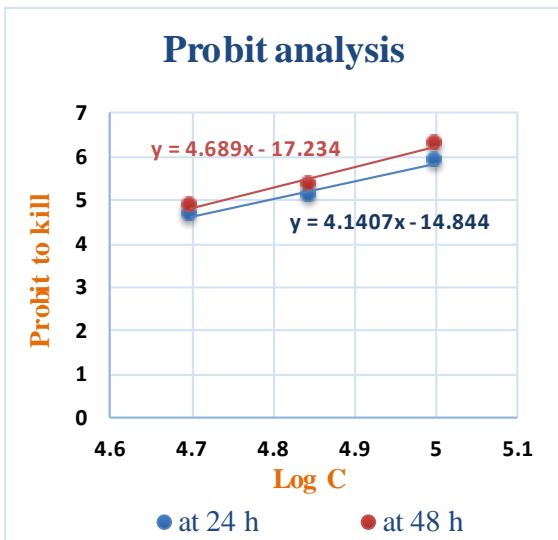


Figure 1 Probit analysis of aqueous seed kernel extracts against *S. orientalis*

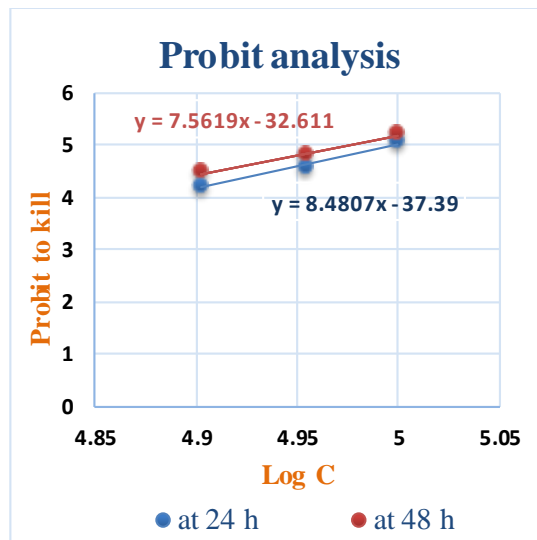


Figure 2 Probit analysis of diethyl ether seed kernel extracts against *S. orientalis*

4.3.3 Relative efficiency of different colored traps with or without bulbs against *S. orientalis*

Five different colored sticky traps (yellow, blue, green, red and white) of size 10×20 cm with bulbs of 0.5 Watt and without bulbs were placed inside the rooms to test their efficacy in catching *S. orientalis*. Observations on color of the trap preferred by the adult flies was recorded after seven days of installation of traps and number of fly stuck on each trap was counted.

Data presented in Table 4 and Figure 3 revealed that, seven days after installation of traps, the maximum catch of 124.67 flies was observed with yellow colored sticky traps without bulbs followed by 73.33 flies on blue sticky traps and 56.33 flies on green sticky traps. The white and red traps were least attractive to adults of this fly. In case of light traps the number of sciarid flies trapped with white bulb (118.33 flies) was significantly higher than any other bulb tested, followed by yellow colored bulb with 115 flies and blue bulb with 82.67 flies. The minimum number of flies was caught by red trap (24 flies) followed by green trap with 49.33 flies.

Table 4. Efficacy of different colored sticky traps and light traps in catching *S. orientalis*

Traps	Traps with bulbs	Traps without bulbs	Mean
Yellow Trap	115.00	124.67	119.83
Blue trap	82.67	73.33	78.00
Green trap	49.33	56.33	52.83
Red trap	24.00	33.67	28.83
White trap	118.33	17.33	67.83
Mean B	77.87	61.07	

CD _{0.05}	
Traps (T)	6.64
Bulbs (B)	4.19
T × B	9.39

Soni supported the present investigations as he evaluated four different colored light traps along with one plain bulb to manage sciarid fly at Raipur [13]. He concluded that plain bulb had the maximum attraction capacity of 259.4 flies per day as compared to any other colored bulb. After plain bulb, next preferred light trap was of yellow color followed by green colored bulb. He observed that orange and red bulbs were least preferred at all. These results are further supported by the findings of Gorska-Drabik et al. who evaluated yellow and blue colored traps for their efficacy in catching sciarid flies and concluded that yellow colored traps caught 67.00 per cent flies and blue traps caught remaining 33.00 per cent flies [10].

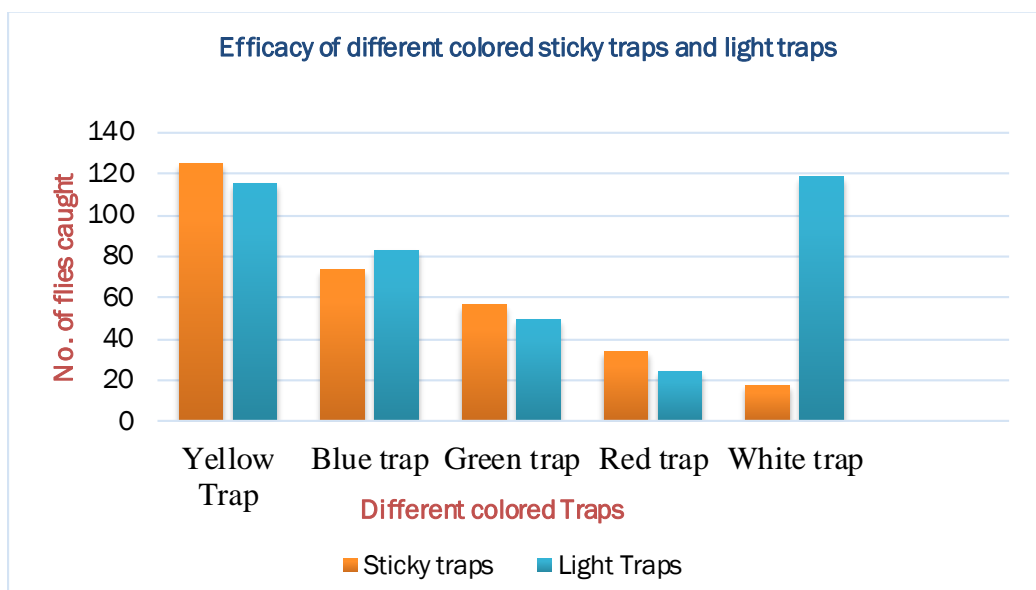


Figure 3 Efficacy of different colored sticky traps and light traps

The present findings were in accordance with the findings of Sahin et al. (2016): in his studies, he tested the efficacy of three colored traps viz., yellow, blue and white in capturing the mushroom fly on *A. bisporus* and concluded that yellow traps were best in capturing sciarid fly followed by blue colored traps and least attraction was shown by white traps. Bingham (2004) also reported that the adult flies caught on light traps were more in number as compared to sticky traps.

3.3 Repellent property of aqueous, ethanol and diethyl ether seed kernel extracts of *M. azedarach* against *S. orientalis*

Aqueous, ethanol and diethyl ether seed kernel extracts of *M. azedarach* was tested for their repellent effect against *S. orientalis*. The data on per cent repellency shown by different extracts of *M. azedarach* to *S. orientalis* has been given in Table 5. Data presented in Table 5 revealed that the highest mean per cent repellency of 66.47 was shown

by diethyl ether seed kernel extracts at 10.0 per cent concentration followed by ethanol seed kernel extract at same concentration after 24 h. The minimum per cent repellency of 39.97 was shown by seed kernel extracts of *M. azedarach* at 10.0 per cent concentration after an exposure period of 24 h.

Both leaf and seed kernel extracts at 2.0 per cent concentration were tested by Nathan et al. (2006) and results showed that with the increase in concentration, the repellency increased and it was slightly higher in seed extracts as compared to the leaf extracts. Dade et al. (2018) tested repellency of seed kernel extracts of *M. azedarach* against *Triatoma infestans* after 24 h of exposure period. He concluded that repellent property was directly proportional to the concentration of extracts. Farag et al. (2011) also reported that repellent property of fruit extracts of *M. azedarach* increased with increase in concentration.

Table 5. Per cent repellent rate of seed kernel extracts of *M. azedarach* on adults of *S. orientalis* after an exposure of 24 h

Treatments	Per cent repellent rate after an exposure of 24 h
Aqueous seed kernel extract of <i>M. azedarach</i>	39.97 ± 4.99
Ethanol seed kernel extract of <i>M. azedarach</i>	48.99 ± 8.76
Diethyl ether seed kernel extract of <i>M. azedarach</i>	66.47 ± 3.68
	N/A
CD _{0.05}	6.20
SE (d)	8.76
SE (m)	20.72
C.V.	

Conclusion

Effect of seed kernel extracts (aqueous, ethanolic and diethyl ether) of *Melia azedarach* and agniastra (2.0 %) was evaluated for larval mortality, repellency and mycelial inhibition. The aqueous extracts and agniastra were found effective against *S. orientalis* and caused 0.00 per cent mycelial inhibition to *A. bisporus*. Out of all the concentration tested against *S. orientalis*, maximum per larval mortality of 85.84 per cent was attained at 10.0 per cent concentration of aqueous seed kernel extracts followed by agniastra (67.50 %) at 2.0 per cent concentration against *S. orientalis*. Different colored traps with and without bulbs were also tested for their efficacy against *S. orientalis*, out of which yellow traps without bulb and white traps with bulb were found most efficient in catching flies,. Among seed kernel extracts, diethyl ether extracts showed maximum per cent repellency.

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