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Efficacy of *Mentha piperita* against *Suidasia nesbitti* Hughes (Acari: Suidasiidae) in Stored Bengal Gram

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ABSTRACT

Mentha a member of the Lamiaceae family is recognized for its vital oils, medicinal uses, insecticidal activity and miticidal action. Investigations on the effects of aqueous and methanolic extract of *Mentha pipertica* on *Suidasia nesbitti* Hughes (Acari: Suidasiidae) in stored Bengal gram showed 100 % mite mortality at 4% and 8 % of aqueous extract after 45 and 30 days of post treatment. No mites were recovered from methanolic extract (4% and 8%) within 45 and 15 days of treatment. Amongst the two extracts, methanolic extract was more effective against *S. nesbitti* because of lower LC_{50} value (1.15%) than aqueous extract of *M. pipertica* (1.74%) under direct spray bioassay. With increase in aqueous and methanolic extract concentration from 0.58 %, esterase activity of *S. nesbitti* increased significantly from 1.13 to 2.73OD m^{-1} and 1.60 to 3.79 OD m^{-1}, respectively. It showed significantly greater activity of esterase at higher concentrations after 48 h as compared to lower concentrations. The Glutathione-S-transferase activity in *S. nesbitti* also increased with increase in exposure time of extract. It was 0.005OD m^{-1} after 24 h which increased to 0.006OD m^{-1} after 48 h of exposure with aqueous extract and from 0.005 to 0.006 OD $m^{-1}$with methanolic extract. The Glutathione-S-transferase activity in *S. nesbitti* also increased with increase in exposure time of aqueous and methanolic extract of *M. pipertica* although the increase was non-significant.

Key words: *Esterase, Glutathione-S-transferase, Mentha pipertica, Suidasia nesbitti*

Introduction

Bengal gram (*Cicer arietinum* L.) is a chief pulse crop of Rabi season belongs to the family Leguminaceae or Fabaceae and grown in about 6.67 m ha area, which is 30% of the total area in India. India is the largest Bengal gram (66.19%) producing country in the world (Anonymous, 2019). Bengal gram is a good source of minerals, proteins, carbohydrates and unsaturated fatty acids. Its grains contain 1822 % protein, 5270 % carbohydrate, 4-6% fat 8.51% moisture, 2.72% ash and 2.60% crude fibre (Ahlawat *et al.,* 1981; Alane *et al.,* 2021). In addition to its high protein content, Bengal gram is a good source of antioxidant properties and low glycaemic index (Fredriksson *et al.,* 2000; Crujeiras *et al*., 2007). During storage, these grains are attacked by mites particularly in areas with high relative humidity and high temperature. Among these, *Suidasia nesbitti* is a predominant mite which can reduce the quality and quantity of a food product directly via damage through feeding (Armitage *et al.,* 2002) and seriously endanger human health. Devi *et al.* (2022a) observed that *S. nesbitti* infestation decreased the total soluble sugars, non- reducing sugars, starch and protein content of Bengal gram with increase in observation

period. The weight loss due to *S. nesbitti* infestation in Desichanna was 3.21% in flour form, 2.50% in broken grains and 1.36% whole grains (Devi *et al.,* 2022b).

Synthetic pesticides have been applied to protect the stored grains and other agricultural products from insect infestation, but they caused environmental pollution, food contamination and mortality in non-target organisms (Gupta *et al.,* 2001; Grella *et al*., 2020, Vieira *et al.,* 2020; Ghaste *et al.,* 2020). As a result, many workers advocated the use of Integrated Pest Management strategy to protect the stored grains (Stejskal *et al.,* 2019). Several studies have been carried out on the use of botanicals such as essential oils and their bioactive chemical constituent as an alternative to synthetic insecticides because many plant extracts possess insecticidal efficacy, quick disintegration and less bioaccumulation, as well as low toxicity to non-target organisms (Rajendran and Srianjini, 2008; Chu *et al*., 2011, Grdisa and Grsic, 2013). *Mentha* leaf powder was effective in decreasing the fecundity of *T. putrescentiae* (Schrank) and reducing the mean egg numbers to 25.49 female^{-1} as compared to 98.16 egg female^{-1} in the control (Gulati and Mathur, 1995). The essential oil (Menthol, pulegone, carvoneand menthone) of *M. piperita* exhibited acaricidal activity against *Tyrophagouslongior* (Mimica-Dukic *et al.,* 2003; Kasrati *et al.,* 2015). Ali *et al*. (2022) used botanical sprays and concluded that they delayed the use of chemical pesticide on cotton crop for up to 80–85 days. Aqueous extract of *Glycyrrhizaglabra* and *Ocimum sanctum* provided 71.5 to 94.7 and 66 to 92percent protection against mites in stored wheat at different durations (Anita *et al.* 2014a). Mangoba and Alvindia (2019) reported 100 percent mortality in *S. pontifica*due to garlic crude extract at 0.75 - 1.0 g/l. Essential oils of oregano, thyme, lavender and mint were found to cause 100% adult mortality of *T. cinnabarinus* in a concentration dependent manner (Sertkaya *et al*. 2010). Kavallieratos *et al.* (2022) observed that *M. longifolia caused* 87.8 and 67.8 % mortality in *A. siro* adults and nymphs, respectively. Therefore, the present study was conducted to ascertain the effectiveness of *M. pipertica* against *Suidasia nesbitti* in stored Bengal gram.

Materials and Methods

During the present study, leaves of *Mentha pipertica* were collected from university campus, thoroughly washed to remove the dirt and cut in to small pieces. The leaves (100 g) were soaked in 100 ml distilled water to prepare aqueous extract and another 100 g leaves were soaked into 100 ml methanol for 48 h with sporadic shaking to prepare methanolic extract. After 48 h, both the solutions of *M. pipertica* were filtered through the muslin cloth separately and collected in separate beakers. The filtrates were considered as crude aqueous and methanolic extracts. Further dilutions of 0.5%, 1%, 2%, 4% and 8% were prepared from these 2 stock solutions following volume to volume dilution method. The experiment was completed in six month.

For each extract, 6 sub sets of various concentrations were evaluated against adult mites of *S. nesbitti* under triplicate conditions. In each replicate, 20 mites were released 1 g^{-1} grains. Under direct spray bioassay, grains with mites were sprayed with required concentration. Uninfested Bengal gram grains acted as control in each set on which no spray was done (0%). Observations on the number of live mites in each replicate of sub sets of aqueous and methanolic extracts of *M. pipertica* were recorded after 1, 2, 3, 4, 5, 6, 7,15, 30 and 45 days of treatment. Immobile *S. nesbitti* mites were explored with bird feather's pick, if they failed to respond with leg movement, they were considered as dead. The observed mortality was converted into % mortality and corrected mortality was obtained after subtracting the mortality of *S. nesbitti* in control treatments. The observed data was used for the calculation of LC_{50}. The % mortality of mites was calculated by the following formula:

$$\% \text{ Mortality} = \frac{\text{Total number of mites died}}{\text{Total number of initial mites}} \times 100 \quad ..(1)$$

Acaricidal activity of aqueous and methanolic extracts of *M. pipertica* were evaluated against *S. nesbitti* in stored Bengal gram grains under *in vitro* conditions at 27±1° C and 8085 % relative humidity. The effect of these extracts on enzymatic activity of *S. nesbitti* were also studied.

Results and Discussion

Acaricidal activity of aqueous extract of *Mentha pipertica*

The results on the acaricidal activity of aqueous extract of *M. pipertica* against *S. nesbitti* on Bengal gram grains revealed significant effect of concentration of extract and observation period.

Maximum number of mites (41.06 mites) was recovered from untreated (0%) Bengal gram grains which significantly decreased to 17.86, 14.03, 12.03, 4.87, 3.73 mites at 0.5%, 1%, 2%, 4% and 8% concentration (CD=0.10; p=0.05). Duration wise, the number of mites continuously and significantly decreased up to six days of treatment (CD=0.14; p=0.05). *S. nesbitti* number was 16.67, 15.61,14.00, 13.16, 12.00 and 11.44 mites after 1, 2, 3, 4, 5 and 6 days of post treatment. Afterwards, the number of mites significantly increased to 13.11, 15.89, 19.89 and 24.22 mites after 7, 15, 30 and 45 days of treatment (Table 1). Significant interaction was observed between the treatments and observation periods (CD=0.34; p=0.05) (Table 1) which indicated that higher concentrations were significantly more effective than lower concentrations at all durations.

Acaricidal activity of methanolic extract of *Mentha pipertica*

The data pertains to acaricidal activity of methanolic extract of *Mentha pipertica* against *S. nesbitti* on Bengal gram grains is presented in Table 2.

The untreated grains harboured significantly greater number of *S. nesbitti* g^{-1} grains (32.96 mites) as compared to treated grains (CD=0.17; p=0.05). Higher concentrations were more effective in reducing the number of live mites as compared to lower concentrations. With an initial inoculums of 20 mites, significantly less number of mites were recorded at 8% (2.90 mites), 4% (4.13 mites) concentrations than at 2% (8.57 mites), 1% (10.23 mites) and 0.5% (13.56 mites) concentration and control. Duration of the treatment showed significant reduction in *S. nesbitti* population to 15.05, 13.50, 11.89, 10.39, 9.50, 8.55, 8.83, 10.66, 13.83 and 18.39 mites after 1, 2, 3, 4, 5, 6, 7, 15, 30 and 45 days of treatment from the initial 20 mites (CD=0.23; p=0.05). Statistically significant interaction between the treatments and observation periods was recorded (CD=0.56; p=0.05) which indicated that all the treatments were significantly better than the control treatment. Methanolic extract at 8% concentration caused 100 % mortality within 15 days of treatment whereas, no mites were recovered from 4 % treated Bengal gram grains after 45 days of treatment. Similar results were reported by Gulati and Mathur (1995) who recorded 100 % mortality with mint oil. The survival rate and fecundity of *T. urticae* were both drastically lowered by Lavandula latifolia essential oil (Laborda *et al.*, 2018).

Table 1. *In vitro* bioassay aqueous extract of *Menthapipertica* against *Suidasianesbitti*

Treatments (%)	Pre treatment count	Average number of mites after days of treatment										Mean
		1d	2d	3d	4d	5d	6d	7d	15d	30d	45d	
0	20.00	23.66	266.6	29.66	356.6	39.00	43.00	47.00	51.66	55.33	59.00	41.06
		(4.96)	(5.25)	(5.53)	(6.05)	(6.32)	(6.63)	(6.92)	(7.25)	(7.50)	(7.74)	(6.41)
0.5	20.00	19.33	18.00	16.00	13.00	9.66	8.00	10.66	18.66	27.00	38.33	17.86
		(4.50)	(4.35)	(4.12)	(3.74)	(3.26)	(2.99)	(3.41)	(4.42)	(5.28)	(6.27)	(4.23)
1.0	20.00	17.00	16.00	13.00	10.66	9.00	7.00	8.66	12.66	20.33	26.00	14.03
		(4.24)	(4.12)	(3.74)	(3.41)	(3.15)	(2.81)	(3.10)	(3.69)	(4.61)	(5.19)	(3.80)
2.0	20.00	16.00	14.00	10.66	8.66	6.66	5.00	10.00	11.00	16.33	22.00	12.03
		(4.12)	(3.87)	(3.41)	(3.10)	(2.75)	(2.44)	(3.31)	(3.45)	(4.15)	(4.78)	(3.53)
4.0	20.00	13.00	11.00	8.33	6.00	4.00	3.33	1.66	1.00	0.33	0.00	4.87
		(3.74)	(3.46)	(3.05)	(2.64)	(2.22)	(2.07)	(1.62)	(1.38)	(1.13)	(1.00)	(2.23)
8.0	20.00	11.00	8.00	6.33	5.00	3.66	2.33	0.66	0.33	0.00	0.00	3.73
		(3.46)	(2.99)	(2.69)	(2.44)	(2.15)	(1.82)	(1.27)	(1.13)	(1.00)	(1.00)	(1.99)
Mean	20.00	16.67	15.61	14.00	13.16	12.00	11.44	13.11	15.89	19.89	24.22	
		(4.17)	(4.00)	(3.75)	(3.56)	(3.30)	(3.12)	(3.27)	(3.55)	(3.94)	(4.33)	

Figures in parentheses are √n+1 transformation values
CD (p=0.05) for Treatment = (0.10); SE (m) = (0.03)
CD (p=0.05) for Days of Treatment = (0.14); SE (m) = (0.05)
CD (p=0.05) for Treatment × Days of Treatment = (0.34); SE (m) = (0.12)

Table 2. *In vitro* bioassay of Methanolic extract of *Menthapipertica* against *Suidasianesbitti*

Treatments (%)	Pre treatment count	Average number of mites after days of treatment										Mean
		1d	2d	3d	4d	5d	6d	7d	15d	30d	45d	
0	20.00	21.66	23.66	24.66	27.66	30.33	33.33	35.66	37.66	43.33	51.66	32.96
		(4.75)	(4.96)	(5.06)	(5.35)	(5.59)	(5.85)	(6.05)	(6.21)	(6.30)	(7.08)	(5.72)
0.5	20.00	19.00	15.66	13.66	10.66	8.66	6.33	7.00	12.00	17.66	25.00	13.56
		(4.47)	(4.08)	(3.82)	(3.40)	(3.09)	(2.68)	(2.82)	(3.58)	(4.30)	(5.08)	(3.73)
1.0	20.00	16.00	14.33	11.66	9.00	6.66	4.33	3.66	7.66	11.33	17.66	10.23
		(4.11)	(3.90)	(3.55)	(3.15)	(2.74)	(2.27)	(2.15)	(2.93)	(3.50)	(4.30)	(3.26)
2.0	20.00	13.33	11.00	9.00	7.00	5.33	3.33	4.33	6.00	10.33	16.00	8.57
		(3.78)	(3.45)	(3.15)	(2.81)	(2.50)	(2.06)	(2.26)	(2.48)	(3.29)	(4.07)	(2.98)
4.0	20.00	11.00	9.33	7.33	5.00	3.66	2.33	1.66	0.66	0.33	0.00	4.13
		(3.45)	(3.20)	(2.87)	(2.42)	(2.13)	(1.80)	(1.62)	(1.27)	(1.13)	(1.00)	(2.08)
8.0	20.00	9.33	7.00	5.00	3.00	2.33	1.66	0.66	0.00	0.00	0.00	2.90
		(3.20)	(2.82)	(2.44)	(1.98)	(1.82)	(1.62)	(1.27)	(1.00)	(1.00)	(1.00)	(1.81)
Mean	20.00	15.05	13.50	11.89	10.39	9.50	8.55	8.83	10.66	13.83	18.39	
		(3.96)	(3.73)	(3.48)	(3.18)	(2.97)	(2.71)	(2.69)	(2.91)	(3.25)	(3.75)	

Figures in parentheses are √n+1 transformation values CD (p=0.05) for Treatment = (0.17); SE (m) = (0.06) CD (p=0.05) for Days of Treatment = (0.23); SE (m) = (0.08) CD (p=0.05) for Treatment × Days of Treatment = (0.56); SE (m) = 0.20

LC_{50} values of *Mentha pipertica* extracts for *Suidasia nesbitti*

Amongst the two extracts, methanolic extract of *M. pipertica* was more effective against *S. nesbitti* because of lower LC_{50} value (1.15%) than aqueous extract of *M. pipertica* (1.74%) under direct spray bioassay Table 3.

The value of slope was 0.46 and 0.62 for methanolic and aqueous extracts. These lesser values for both the extracts showed that further increase in concentrations will lead to significant decrease in number of *S. nesbitti* in Bengal gram grains. Zandi-Sohani and Ramezani, 2015 used the five essential oils of *Saturejahortensis*, *Menthapulegium*, *Menthaviridis*, *Rosemarinus officinalis* and *Zatariamultiflora* on the mortality of adult females of Tetranychusturkestani and found the mortality of females increased with increasing concentrations of essential oils and exposure times.

Detoxification enzyme bioassay studies

Effect of aqueous extract of *M. pipertica* on esterase activity of *S. nesbitti* showed significantly lower values of esterase (1.01 OD m^{-1}) in mites feeding on untreated (0%) Bengal gram grains as compared to treated grains (CD= 0.03; p=0.05) Table 4.

Table 4. Effect of aqueous extract of *Mentha pipertica* on esterase activity of *Suidasia nesbitti*

Treatments (%)	Esterase activity (OD m^{-1})		Mean
	24 h	48 h	
0	0.73	0.75	1.01
0.5	0.93	1.33	1.13
1.0	1.13	1.46	1.29
2.0	1.66	1.86	1.76
4.0	1.93	2.66	2.29
8.0	2.53	2.93	2.73
Mean	1.48	1.92	

CD (p=0.05) for Treatment = 0.03; SE (m) = 0.01CD (p=0.05) for Duration = 0.02; SE (m) = 0.01CD (p=0.05) for Treatment × Duration=0.05; SE (m) =0.02

With increase in aqueous extract concentration from 0.58%, esterase activity of *S. nesbitti* increased significantly from 1.132.73 OD m^{-1}. Esterase activity was 1.48 OD m^{-1} after 24 h of spray which significantly increased to 1.92 OD m^{-1} after 48 h of spray (CD=0.02; p=0.05). It showed significantly greater activity of esterase at higher concentrations after 48

Table 3. LC_{50} values of *Mentha pipertica* extracts for *Suidasia nesbitti*

Mentha pipertica leaf extracts	Direct spray bioassay					
	n	Slope	Intercept	LC_{50} (%)	÷2	Df
Aqueous extract	6	0.62	4.59	1.74	1.04	5
Methanolic extract	6	0.46	4.06	1.15	1.38	5

h as compared to lower concentrations.

The Glutathione-S-transferase activity in *S. nesbitti* increased with increase in exposure time of aqueous extract of *M. pipertica* (Table 5).

Table 5. Effect of aqueous extract of *Mentha pipertica* on Glutathione-S-transferase activity of *Suidasia nesbitti*

Treatments (%)	Glutathione-S-transferase (OD m^{-1})		Mean
	24 h	48 h	
0	0.002	0.002	0.002[a]
0.5	0.003	0.003	0.003[a, b]
1.0	0.004	0.005	0.004[b]
2.0	0.006	0.007	0.006[c]
4.0	0.007	0.008	0.007[c, d]
8.0	0.008	0.009	0.008[d]
Mean	0.005	0.006	

It was 0.005 OD m^{-1} after 24 h which increased to 0.006 OD m^{-1} after 48 h of exposure although the increase was non-significant. The Glutathione-S-transferase activity was statistically at par between 0% and 0.5%, 0.5% and 1%, 2% and 4%, 4% and 8 % concentration. The interaction between Glutathione-S-transferase activity in treated grains and duration was also non-significant.

Similarly, with increase in methanolic extract concentration from 0.58%, esterase activity of *S. nesbitti* significantly from 1.603.79 OD m^{-1} (Table 6).

Table 6. Effect of methanolic extract of *Mentha pipertica* on esterase activity of *Suidasia nesbitti*

Treatments(%)	Esterase activity (OD m^{-1})		Mean
	24 h	48 h	
0	1.13	1.80	1.29
0.5	1.40	1.93	1.60
1.0	1.60	2.80	1.76
2.0	2.06	3.53	2.43
4.0	2.86	4.13	3.19
8.0	3.46	1.46	3.79
Mean	2.08	2.60	

CD (p=0.05) for Treatment = 0.07; SE (m) = 0.02CD (p=0.05) for Duration = 0.04; SE (m) = 0.01CD (p=0.05) for Treatment × Duration =0.09; SE(m)=0.03

Esterase activity was 2.08 OD m^{-1} after 24 h of spray which significantly increased to 2.60 OD m^{-1} after 48 h of spray (CD=0.04; p=0.05). The ANOVA revealed a significant interaction between treatments and duration (CD=0.09; p=0.05). The results on the Glutathione-S-transferase activity in *S. nesbitti* showed that the activity increased with increase in exposure time of methanolic extract of *M. pipertica* (Table 7).

Table 7. Effect of methanolic extract of *Menthapipertica* on Glutathione-S-transferase activity of *Suidasia nesbitti*

Treatments (%)	Glutathione-S-transferase activity (OD m^{-1})		Mean
	24 h	48 h	
0	0.003	0.003	0.003
0.5	0.004	0.004	0.004
1.0	0.005	0.006	0.005
2.0	0.006	0.008	0.007
4.0	0.007	0.009	0.008
8.0	0.008	0.01	0.009
Mean	0.005	0.006	

CD (p=0.05) for Treatment = 0.003; SE (m) = 0.001CD (p=0.05) for Duration = N/A; SE (m) = 0.001CD (p=0.05) for Treatment×Duration=N/A; SE(m)=0.002

It was 0.005OD m^{-1} after 24 h which increased to 0.006 OD m^{-1} after 48 h of exposure although the increase was non-significant. Otero *et al.* (2018) investigated the effects of essential oil from *Cymbopogon flexuosus* (Cochin grass) on GST and nonspecific esterases in two *Aedes aegypti* L populations (Rock and WSant). They discovered that high amounts of essential oil inhibited the activities of GST and esterase in the Rock population. The essential oil of *Atalantia monophylla* also had a substantial impact on the protein, esterase, and GST of *Callosobruchus maculatus* F. and *Sitophilus oryzae* L. (Nattudurai *et al.*, 2017). Furthermore, numerous studies have been published that reveal the impact of essential oils on insect detoxification enzymes (Liao *et al.*, 2017; Gao *et al.*, 2019).

Conclusion

The present study showed the acaricidal action of aqueous and methanolic extract of *Mentha pipertica* on *Suidasia nesbitti* Hughes (Acari: Suidasiidae) in stored Bengal gram. On the basis of LC_{50} values, methanolic extract was more effective against mites. Treatment with *M. pipertica* increased the esterase and Glutathione-S-transferase activity of *S. nesbitti*.

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