Insecticidal and oviposition deterrent properties of some spices against coleopteran beetle, *Sitophilus oryzae*

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Abstract Eighteen spices were screened for their insecticidal potential and antiovipositional properties against the rice weevil- *Sitophilus oryzae*—a serious insect pest of stored food grains. The bioefficacy of the powders and hexane extracts of selected spices were determined by assessing the toxicity, effect on F₁ progeny, contact toxicity, persistence and seed viability. Responses varied with the spices, dosage and exposure time. Powders of mace and pepper, at 1% level were highly effective resulting in total mortality of *S. oryzae* by one week followed by nutmeg and clove with 100% mortality and cinnamon and star anise with 90% mortality at 5% concentration. These spices also completely inhibited F₁ progeny. The hexane extracts of these spices at 1,000 ppm showed insecticidal activity, with pepper extract recording 100% mortality by 5 days. *S. oryzae* was susceptible to clove oil resulting in 92% mortality. Mortality was 51.63% in nutmeg, 66.6% in cinnamon, and 79.8% in case of mace and star anise. Hexane extracts of star anise, cinnamon and clove at 0.59 µl/cm² on filter paper discs induced 100% mortality by 72 h. These spices offered protection to wheat up to 9 months without affecting seed germination thereby showing promise as grain protectants.

Keywords *Sitophilus oryzae* · Spices · Insecticidal activity · Antioviposition · Grain protectant · Persistence

Introduction

Sitophilus oryzae L. (Rice weevil)—one of the major insect pests of stored food grains especially of cereals and their products contribute significantly to the physical, nutritional and quality deterioration of food grains. Controlling insect infestation in storage units generally relies on the use of fumigants and residual contact insecticides. However, the implications of these on the health and environment have revived interest in the development of ‘safer compounds’ as grain protectants using the knowledge base of plants. (George et al. 1999; Rajendran and Sriranjini 2008 and Rajashekar et al. 2010)

The essential oils of some spices are reported to exhibit insecticidal activity, (Owolabi et al. 2009). However, knowledge on their application as insecticidal, antifeedant or antiovipositional agent is limited. Hence powders of 18 spices such as mace, pepper, nutmeg, cloves, cinnamon, star anise, fennel (saunf), fennel (Lucknow saunf), ajowan, cumin (jeera), caraway (shah jeera), turmeric, ginger, bay leaves, red chilly, cappers, coriander, and fenugreek were evaluated for their insecticidal, antifeedant and antiovipositional potential against *S. oryzae* infesting wheat. The toxicity of the spice powders to the test insect, effect on oviposition and F₁ progeny, persistence of the treatment on grain and its effect on germination up to 12 months were investigated. The contact toxicity of the hexane extracts of the promising spices were also studied.

Materials and methods

Plant materials The plant materials i.e., 18 different spices listed in Table 1 were procured from the local super market. The spices were ground into fine powder to pass

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Table 1 List of various spices tested against *Sitophilus oryzae*

	Botanical name	Family name	Part used	Yield (%)
Ajowan	<i>Carum copticum</i>	Apiaceae	Seed	28.2
Bay leaves	<i>Laurus nobilis</i> L.	Lauraceae	Leaf	26.2
Cappers	<i>Capparis spinosa</i>	Capparidaceae	Fruit	0.4
Cinnamom	<i>Cinnamomum verum</i>	Lauraceae	Bark, Leaf	4.6
Clove	<i>Syzygium aromaticum</i>	Myrtaceae	Flower bud	38.0
Coriander	<i>Coriandrum sativum</i> L.	Apiaceae	Leaf, Seed	17.0
Cumin (jeera)	<i>Cuminum cyminum</i> L.	Apiaceae	Fruit	18.4
Caraway (shah jeera)	<i>Carum carvi</i>	Apiaceae	Fruit	20.0
Fennel (saunf)	<i>Foeniculum vulgare</i>	Apiaceae	Seed	18.6
Fennel (Lucknow saunf)	<i>Foeniculum vulgare</i>	Apiaceae	Seed	19.4
Fenu greek	<i>Trigonella foenum-graecum</i>	Fabaceae	Fruit	5.8
Ginger	<i>Zingiber officinale</i>	Zingiberaceae	Rhizome	3.6
Mace	<i>Myristica fragrans</i>	Myristicaceae	Flower	28.8
Nutmeg	<i>Myristica fragrans</i>	Myristicaceae	Seed	42.8
Pepper	<i>Piper nigrum</i>	Piperaceae	Fruit	6.8
Red chilly	<i>Capsicum annum</i> L.,	Solanaceae	Fruit	13.6
Star anise	<i>Illicium verum</i>	Illiciaceae	Dried fruit	6.8
Turmeric	<i>Curcuma longa</i> L.	Zingiberaceae	Rhizome	3.6

through 80 mesh sieve, and extracted in hexane (AR grade) for 48 h. The solvent was evaporated under vacuum in a rotary evaporator. The yield of each extract is given in Table 1

Test insects *S. oryzae* used were drawn from laboratory cultures, reared on whole wheat and maintained at $30\pm 2^{\circ}\text{C}$ and 65–70% RH without exposure to any insecticides.

Insecticidal activity of spice powders Bioassay was performed on adult *S. oryzae*, to determine the activity of the substance when directly applied on grains. Samples of 15 g disinfested wheat were taken in 100×25 mm glass tubes and treated with the required quantities of spice powders to give 1 and 5% (wt/wt) concentrations. The tubes were stoppered and shaken well to obtain uniform adherence of powders to grain surface. Four replicates were maintained for each treatment including control. Into each replicate, 15 *S. oryzae* adults (10–12 days old) were released, and covered with cloth held by rubber band. The mortality counts of insects were recorded periodically up to 21 days. Based on their efficacy the spices were grouped as A, B and C (Table 2).

Insecticidal activity of extracts In another set of experiments, hexane extracts of spice powders from Group A were evaluated for their insecticidal activity. Fifteen grams samples of conditioned wheat were mixed with each of the spice extracts taken in hexane so as to make the final concentration of 1,000 ppm. The treated grains were aerated well and distributed into tubes before releasing the

Table 2 Insecticidal activity of spice powders against *Sitophilus oryzae*

Spices	Mortality % ^a	
	1%	5% [*]
Ajowan	48.3 $\pm$ 7.71	76.6 $\pm$ 5.35
Bay leaves	34.9 $\pm$ 5.85	21.6 $\pm$ 4.63
Cappers	16.6 $\pm$ 1.79	38.3 $\pm$ 5.28
Cinnamon	33.3 $\pm$ 7.55	89.8 $\pm$ 4.02
Clove	59.4 $\pm$ 8.66	100.0 $\pm$ 0.00
Coriander	3.3 $\pm$ 1.76	13.3 $\pm$ 3.58
Cumin (jeera)	11.6 $\pm$ 2.97	34.9 $\pm$ 3.88
Caraway (shah jeera)	23.6 $\pm$ 2.98	68.3 $\pm$ 2.95
Fennel (saunf)	1.7 $\pm$ 1.53	49.9 $\pm$ 3.99
Fennel (Lucknow saunf)	11.6 $\pm$ 1.55	86.6 $\pm$ 5.05
Fenugreek	5.0 $\pm$ 4.63	16.6 $\pm$ 5.35
Ginger	18.6 $\pm$ 3.03	22.2 $\pm$ 5.07
Mace	100.0 $\pm$ 0.00	100.0 $\pm$ 0.00
Nutmeg	74.7 $\pm$ 4.47	100.0 $\pm$ 0.00
Pepper	100.0 $\pm$ 0.00	100.0 $\pm$ 0.00
Red chilly	4.9 $\pm$ 2.95	16.6 $\pm$ 1.79
Star anise	46.6 $\pm$ 2.51	89.7 $\pm$ 1.92
Turmeric	14.9 $\pm$ 1.55	31.6 $\pm$ 6.35

^{*} Group A: Highly effective with 85–100% mortality- mace, pepper, clove, nutmeg, cinnamon and star anise

Group B: Moderately effective with 50–85% mortality—fennel (Lucknow saunf), ajowan, caraway (shah jeera) and fennel (saunf)

Group C: Less effective with less than 50% mortality cumin (jeera), cappers, turmeric, ginger, bay leaves, fenugreek, red chilly and coriander

a=mean $\pm$ SE, (n=4)

adult insects. Four replicates were maintained for each treatment including control. Observations with reference to adult mortality and F_1 progeny were recorded.

F_1 progeny The emergence of F_1 progeny were recorded for 30 days post emergence. Percentage reduction in emergence of F_1 adults or inhibition rate percentage (IR %) was calculated as $IR \% = (C_n - T_n) 100 / C_n$, where C_n is the number of newly emerged insects in the untreated control samples and T_n is the number of insects emerged in the treated samples (Tapondjou et al. 2002).

Filter paper contact toxicity The contact toxicity of hexane extracts of spices against *S. oryzae* were evaluated on filter paper discs (What man No.1, 9 cm diameter) according to the method described by (Tapondjou et al. 2005). Each treatment consisted of four replicates. Insect mortalities were recorded daily up to 72 h and percent mortality was determined.

Antioviposition activity Oviposition by *S. oryzae* on wheat treated with group A spice powders at 5% level as well as untreated control samples were recorded after staining the grains for egg plugs (Holloway 1985). The wheat grains were also examined for antifeedant activity.

Seed germination Germination test for seed viability of the treated wheat grains was conducted according to the International seed testing methods (Nikpay 2007).

Statistical analysis All the experiments had four replicates ($n=4$) and the data was analysed using ANOVA ($P<0.05$) and expressed as means $\pm$ SE. LD_{50} values were calculated by probit analysis (Finney 1971) using Stat plus 2008 software.

Results and discussion

Insecticidal activity of spice powders Mortality of the weevil exposed to each of the 18 spices on wheat at 1% and 5% levels is shown in Table 2. Significant differences in toxicity of the test materials were observed with pepper and mace ($P<0.05$) causing 100% mortality at both the concentrations tested by 1 week. Powders of nutmeg and clove at 5% level killed 100% insects by 14 days. Insecticidal action with cinnamon, star anise, fennel (Lucknow saunf), ajowan and caraway (shah jeera) was represented by 89.8, 89.7, 83.6, 76.6 and 68.3% mortality of *S. oryzae*, respectively. There was an increase in mortality from 1% to 5% level for other spices with fennel (Lucknow saunf) showing a remarkable performance from

11.6% to 86.6% mortality. Weak activity was observed with coriander, red chilly, fenugreek, ginger, bay leaves and turmeric with mortality ranging from 13% to 31.6%. Based on their insecticidal potential (at 5% Conc.) the spices were grouped as follows- **Group A:** Highly effective with 85–100% mortality- mace, pepper, clove, nutmeg, cinnamon and star anise. **Group B:** Moderately effective with 50–85% mortality- fennel (Lucknow saunf), ajowan, caraway (shah jeera) and fennel (saunf) and **Group C:** Less effective with 1–50% mortality – cumin (jeera), cappers, turmeric, ginger, bay leaves, fenugreek, red chilly and coriander.

Insecticidal activity of spice extracts The toxicity of extracts of selected spices (Group A) at 1,000 ppm concentration to *S. oryzae* adults exposed to direct contact is shown in the Fig. 1. The extracts of these spices showed insecticidal activity, with pepper oil recording 100% mortality by 5 days. *S. oryzae* was susceptible to clove oil resulting in about 92% mortality by 21 days. With other spice extracts mortality rate was 79.8% (mace and star anise) 66.6% (cinnamon), and 51.63% (nutmeg).

Effect of spice powders on F_1 population Table 3 shows the number of F_1 progeny produced by *S. oryzae* after being exposed to the different spice powders at 1 and 5% concentration. There was a significant ($P<0.05$) difference amongst the treatments over the control. In case of treatments with pepper, nutmeg, mace and clove there was no F_1 emergence even at the lowest concentration tested. Cinnamon and star anise recorded 100% inhibition of F_1 emergence, followed by fennel (Lucknow saunf) with 97.32% inhibition at 5% level. In case of ajowan, bay

Fig. 1 Insecticidal activity of spice extracts at 1000 ppm on *Sitophilus oryzae*. 1. Cinnamon, 2. Clove, 3. Mace, 4. Nutmeg, 5. Pepper, 6. Star anise, 7. Control ($n=4$)

Table 3 Effect of spice powders on F_1 population against *Sitophilus oryzae*

Spices	F_1 Progeny			
	1%		5%	
	a	Inhibition %	a	Inhibition %
Ajowan	72.8±15.28	65.4	37.8±04.18	83.0
Bay leaves	50.0±05.89	76.3	42.3±05.94	81.3
Cappers	181.0±10.25	14.2	195.0±10.34	12.9
Cinnamon	33.3±06.75	84.4	0.0±00.00	100.0
Clove	0.0±00.00	100.0	0.0±00.00	100.0
Coriander	61.8±02.43	70.6	112.5±20.52	49.6
Cumin (jeera)	51.3±04.06	75.8	27.8±04.37	87.5
Caraway (shah jeera)	127.8±09.47	39.3	52.3±08.80	76.8
Fennel (saunf)	104.5±07.60	50.2	23.8±05.24	89.3
Fennel (Lucknow saunf)	43.0±03.00	79.6	6.3±02.34	97.3
Fenugreek	109.5±10.50	47.9	81.8±06.58	63.4
Ginger	129.5±09.52	38.4	112.8±06.17	49.6
Mace	0.0±00.00	100.0	0.0±00.00	100.0
Nutmeg	0.0±00.00	100.0	0.0±00.00	100.0
Pepper	0.0±00.00	100.0	0.0±00.00	100.0
Red chilly	92.3±01.79	56.4	49.0±06.10	78.1
Star anise	117.5±09.90	44.1	0.0±00.00	100.0
Turmeric	198.5±07.26	5.7	76.5±07.49	65.6

a= F_1 number mean±SE, (n=4)

leaves, cumin (jeera), caraway (shah jeera), red chilly and fennel (saunf). F_1 emergence was reduced by more than 75%. However, turmeric and cappers affected progeny development minimally with only 5.68% and 14.21% inhibition at 1% level.

Contact toxicity The exposure of *S. oryzae* to each of the spice oils up to 72 h on filter paper discs showed increased percentage mortality with time. Mortality was almost nil at low concentration (0.39 $\mu\text{l}/\text{cm}^2$) of the essential oils during the first 24 h after treatment but increased rapidly thereafter. The results of probit analysis (Table 4) showed that *S. oryzae* was comparatively more susceptible to the toxic effect of clove, cinnamon, mace and star anise by 48 h. It was not possible to estimate LD_{50} values for 24 h exposure

period due to low mortality and for 72 h due to 100% mortality.

Effect on oviposition Since there was total suppression of F_1 progeny in pepper, mace, nutmeg, cinnamon, clove and star anise, the influence of these spices on oviposition was evaluated. In each of the above spices oviposition was determined by egg plug staining. Egg plugs were nil in these treatments as compared to about 225 to 250 eggs/ 100 grains of wheat in control. Grains treated with Group A spices were found to be intact without any damage. The adult days for mace, pepper, nutmeg, clove, cinnamon and star anise were 18, 23, 40, 52, 67 and 73, respectively.

Residual activity Persistent effect of the selected spice powders of Group A were evaluated by releasing fresh batch of insects after six, 9 and 12 months. Star anise and cinnamon at 5.0% level were effective up to 9 months, whereas pepper, clove, mace and nutmeg at 5.0% were effective for more than a year by retaining their insecticidal activity against *S. oryzae* recording 100% mortality by 15–21 days.

Effect on seed viability Since many of the admixtures of grain protectants affect the seed viability preventing germination, germination tests were carried out with wheat grains treated with spices which exhibited insecticidal activity till 9 months of storage. All the wheat grains

Table 4 Contact toxicity of different spice extracts against *Sitophilus oryzae*

Spice extracts	LD_{50} ($\mu\text{l}/\text{cm}^2$)	SE ($\pm$)
Cinnamon	0.49 (0.44–0.54)	0.03
Clove	0.52 (0.48–0.55)	0.02
Mace	0.58 (0.56–0.62)	0.02
Nutmeg	1.18 (0.96–2.14)	0.24
Pepper	1.00 (0.80–2.08)	0.25
Star anise	0.58 (0.53–0.65)	0.03

Values in parentheses represent lower and upper confidential limits, (n=4)

treated with the test compounds germinated on par with the untreated control grains. Thus the treatments had no inhibitory effect on seed germination.

Spices and their extracts are known to have various effects on stored product insects (Kim and Ahn 2001; Owolabi et al. 2009). Spices have characteristic flavours and odours due to the essential (volatile) oils. Essential oils of spices and their constituents have also been reported as a potent source of botanical pesticides (Singh and Upadhyay 1993). The essential oils of several spices like anise (*Pimpinella anisum* L.) and peppermint (*Mentha piperita* L.) have been found to have fumigant toxicity to four major stored product pests, *R. dominica*, *Tribolium castaneum* (Herbst), *S. oryzae* and *Orzyaephilus surinamensis* (L.) (Shaaya et al. 1991). Ho et al. (1996) found that the essential oil of garlic is insecticidal to *T. castaneum* and *Sitophilus zeamais* Motschulsky. Tripathi et al. (2002) have reported the bioactivities of the leaf essential oil of *Curcuma longa* (var Ch-66) on three species of stored-product beetles.

In this study, powders of 18 spices were tested by direct contact application against adults of *S. oryzae*. The insecticidal activity varied with the spices, concentration and exposure time. Powders of spices of group A i.e., mace, pepper, clove, nutmeg, cinnamon and star anise exhibited potent insecticidal activity than other spices at 1 and 5% concentrations. In these cases, the treated grains were free from infestation due to inhibition of F1 progeny. Though the adult days were significantly higher, there was no damage of treated wheat grains, indicating their antifeedant activity, which has probably resulted in nil oviposition. Thus, our study shows that these spices which act as antifeedants and exhibit antiovipositional and insecticidal properties could be used as potential grain protectants.

Hexane extracts of the same showed their insecticidal potential even at much lower dose. Pepper oil was highly effective recording 100% mortality by 5 days followed by clove with 92% mortality. *S. oryzae* was susceptible to the toxic effect of clove, cinnamon, mace and star anise with LD₅₀ values of 0.517, 0.493, 0.584 and 0.584 µl/cm², respectively as revealed by the contact toxicity studies.

Conclusion

These spices i.e., mace, pepper, clove, nutmeg, cinnamon and star anise further offered protection to wheat up to 9 months of storage without affecting seed germination. The significant mortality induced the insecticidal, antifeedant and antiovipositional properties and the extended period of protection by these spices suggests an excellent grain protectant potential. These results provide a scientific

rationale for the use of spices in post-harvest grain protection. Further research on the isolation and mechanism of action of their active constituents may be promising approaches for the management of insect pests of storage.

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