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POWDERS OF HERBS FOR PROTECTION AGAINST *CALLOSOBRUCHUS MACULATUS* (F.) IN BLACK GRAM

S. V. S. Gopala Swamy* and B. John Wesley

Post Harvest Technology Centre, Bapatla, Guntur District 522 101 Andhra Pradesh
*Email: paulgopal2003@yahoo.com

ABSTRACT

The efficacy of powders of certain herbs for grain protection against pulse bruchid *Callosobruchus maculatus* in stored black gram was evaluated. It was observed that clove (8.0 adults), sweet flag rhizome (6.0 adults), pepper (9.67 adults) and tobacco leaf powders (16.67 adults) were the effective ones as grain mixing at 0.4%. There was reduced adult emergence in these. These powders were further evaluated under dual choice conditions for their repellent effect. The adult emergence and % grain damage at 40 and 80 days after release were observed to be negligible in these. The spice tablets made of sweet flag and clove powders alone or fortified with clove oil when placed on the grain surface offered protection.

Key words: Black gram, storage, bruchid, clove, sweet flag, pepper, tobacco, adult emergence, repellent, grain damage, spice tablets

Pulses are important in diet and these include crops such as bengal gram, black gram, red gram, green gram and lentil (Singh and Navnath, 2018). Realization of self-sufficiency in pulses is only possible through productivity enhancement along with reducing post-harvest losses. Black gram (*Vigna mungo* L.), is the most important pulse crop in Andhra Pradesh (Directorate of Economics and Statistics, 2018) and plays a significant role in different farming systems. The produce is stored in various quantities for various periods by farmers, traders and millers for varied purposes. Pulse beetle *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) infestation is a major problem preventing safe storage of pulses, and use of insecticides or fumigants is to be restricted owing to the associated hazards (Satya et al., 2016). Search for ecofriendly alternatives has thus become inevitable. Mixing of different plant materials such as soap nut leaves, neem leaves or pongamia leaves which act as insect repellents, antifeedants and oviposition deterrents is one of the traditional methods (Sadanand, 2018). Many plant products such as powders and essential oils had been evaluated for their insecticidal properties (Kumar et al., 2016, Vijayaraghavan and Kavitha, 2016; Kumar et al., 2018; Parmar et al., 2018). With this in view, in this study, certain herbal powders were evaluated against pulse bruchid in black gram.

MATERIALS AND METHODS

The experiment was conducted at the Post Harvest Technology Centre, Bapatla, Guntur District, Andhra Pradesh during 2017-18. The efficacy of flower buds of (T_1) clove *(Syzygium aromaticum* L.), dried fruits of (T_2) black pepper *(Piper nigrum* L.) and (T_3) red chilli *(Capsicum annum* L.), rhizomes of (T_4) turmeric (*Curcuma longa* L.) and (T_5) sweet flag (*Acorus calamus* L.), leaves of (T_6) basil *(Ocimum tenuiflorum* L.) and (T_7) murraya (*Murraya koenigii* L.), and latex of (T_8) asafoetida (*Ferula asafoetida* L.), along with others viz., leaves of (T_9) vasaka *(Adathoda vasica* Nees), and (T_{10}) cured tobacco (*Nicotiana tabacum* L.), and seed kernels of (T_{11}) neem *(Azadirachta indica* Juss) was evaluated with an untreated control (T12). All the herbal materials were shade dried, ground into fine powder and preserved in polythene bags kept in desiccators.

The culture of *C. maculatus* was obtained from local godowns and maintained in the laboratory on black gram (variety LBG 752) after disinfestation. About 250 g of grains was taken in plastic jars (750 ml) and 30 newly emerged adults of *C. maculatus* were released into each. After oviposition by females for a week, the adults were removed. The containers were covered with muslin cloth and kept at 27±2°C and 75±2% RH. After 35 to 40 days, the adults emerged from these were utilized for subcultures and bioassays.

For no-choice test, disinfested black gram grain (100 g) was taken in transparent plastic jars of 250 ml capacity. The grain was thoroughly mixed with the herbal powders individually at 0.4% (*w/w*). After treating the grain, five pairs of freshly emerged bruchids were released into each of the plastic jars. Thus, there

were twelve treatments including an untreated control arranged in completely randomized design. The adults of *C. maculatus* that emerged from different treatments were counted at 40 and 80 days of storage. At 40 days, dead insects were separated and removed after counting and live adults were replaced in to the respective jars. Final data had been pooled to get the total population build up in each treatment. Thus, observations on progeny buildup at 40 and 80 days after release of insects and per cent grain damage at 40 and 80 days were recorded.

For dual-choice test, the treatments which were shown higher efficacy were again tested with an intension to understand their mode of action against pulse bruchid. The relative preference of *C. maculatus* to the black gram grain treated with herbal powder and untreated grain was assessed under dual choice condition, where the insects were allowed to choose their preferred grain and the influence of treatment on progeny development was also observed. For this, a "V" shaped olfactometer consisting of a response chamber (16 cm diameter and 8 cm height) made of transparent polypropylene was designed and it was connected by two test chambers (6 cm in diameter and 200 ml capacity) through PVC tubes (12 cm in length). The bruchids when released into response chamber can perceive odours emanating from the head space of the treated and untreated grains. Accordingly, 100 g of treated black gram grains and untreated grains each were taken in the respective labelled test chambers connecting the response chamber and closed tightly with lids. Later 30 newly emerged unsexed adults were released into the response chamber of the olfactometer and were allowed for 24 hr to move into the test chambers through the arms to reach the test material (treated or untreated grains). Thus, eight sets were maintained to have replications of the experiment. Data on the number of insects moved in to the grain, oviposition, adult emergence and grain damage were recorded and Student's *t*- test was performed to analyse the significance of difference.

To evaluate efficacy of spice tablets, 1 g tablets were prepared using a pelletizer in which the powdered material was placed in the well provided on the platform and compressed by tightening the plunger to form a disc shaped tablet. The sweet flag, black pepper and clove powders individually and also in combination with clove oil (200 µl) were used for preparing tablets. Thus, six different spice tablets i.e., (T_1) clove, (T_2) black pepper, (T_3) sweet flag, (T_4) clove + clove oil (200 µl), (T_5) black pepper + clove oil (200 µl) (T_6) sweet flag + clove oil (200 µl) were prepared to test against pulse bruchid. Black gram grain (100 g) was taken in a plastic container and a tablet was kept over the surface of the grain. After releasing five pairs of adults into each container they were tightly closed. Thus, there were seven treatments including an untreated control with three replications arranged in completely randomized design. Data on oviposition at ten days, adult emergence at 40, 80 and 120 days after release, and % grain damage at 120 days recorded and statistically analysed.

RESULTS AND DISCUSSION

Herbal powders

Efficacy: The mean number of adults emerged at 40 days after release of insects from the black gram treated with different herbal powders ranged from 4.33 in sweet flag to 72.67 in basil leaf powder treatments (Table 1). Sweet flag powder treatment which recorded the lowest adult emergence (4.33) was followed by clove (7.33), which was at par with black pepper (8.33) and neem (9.33) and tobacco (11.67) treatments and were significantly superior to rest of the treatments. The mean adult emergence at 80 DAR was significantly less in the treatments of clove (0.67), black pepper (1.33), sweet flag (1.67) and tobacco (5.0) which were at par with each other, followed by neem powder treatment with 56.67 adults which also was significantly less compared to rest of the treatments. When the adults emerged in 80 days was taken into account, the treatments of sweet flag (6.0), clove (8.0), black pepper (9.67), and tobacco (16.67) allowed only significantly less adult emergence and they were at par with each other. Neem powder recorded a total of 66.0 adults. The adult emergence in the untreated control was 301.33 adults, while the leaf powders of vasaka (367.0), murraya (553.33) and basil (646.33) recorded even higher number of adults compared to the control. Similar trend was observed in grain damage with lowest being in clove (0.67) treatment followed by sweet flag (1.33) and black pepper (1.67) and remained at par.

Thus, sweet flag, clove and pepper powder treatments at 0.4% were found to be effective even in 80 days after treatment. Though tobacco and neem kernel powders were found to suppress adult population and minimize grain damage significantly, as observed with tobacco powder at 0.4% (Swamy and Wesley, 2017) and with neem seed kernel powder at 1% in blackgram (Vijayaraghavan and Kavitha, 2016); owing to known mammalian toxicity of tobacco and

Table 1. Efficacy of herbal powders against C. maculatus in black gram

Tr. No.	Treatment @ 0.4%	Adult emergence (No.) 40 DAR	80 DAR	Total	Grain damage (%) 80 DAR
T_1	Clove	7.33 (2.68)^{b}	0.67 (1.05)^{a}	8.0 (2.79)^{a}	0.67 (3.98)^{a}
T_2	Black pepper	8.33 (2.88)^{bc}	1.33 (1.35)^{a}	9.67 (3.10)^{a}	1.67 (7.33)^{ab}
T_3	Chilli	58.67 (7.65)^{h}	271.33 (16.45)^{d}	330.0 (18.13)^{de}	37.33 (37.65)^{f}
T_4	Turmeric	18.67 (4.31)^{e}	201.0 (14.16)^{c}	219.67 (14.77)^{c}	21.67 (27.62)^{d}
T_5	Sweet flag	4.33 (2.08)^{a}	1.67 (1.46)^{a}	6.0 (2.44)^{a}	1.33 (6.53)^{ab}
T_6	Basil	72.67 (8.51)^{i}	573.67 (23.94)^{f}	646.33 (25.42)^{g}	51.33 (45.77)^{g}
T_7	Murraya	46.33 (6.79)^{g}	507.0 (22.48)^{f}	553.33 (23.48)^{f}	48.67 (44.23)^{g}
T_8	Asafoetida	14.67 (3.83)^{de}	175.67 (13.26)^{c}	190.33 (13.79)^{c}	12.0 (20.25)^{c}
T_9	Vasaka	25.67 (5.05)^{f}	341.33 (18.48)^{e}	367.0 (19.15)^{e}	30.0 (33.21)^{e}
T_{10}	Tobacco	11.67 (3.41)^{cd}	5.0 (2.34)^{a}	16.67 (4.08)^{a}	2.0 (8.13)^{b}
T_{11}	Neem	9.33 (3.06)^{bc}	56.67 (7.54)^{b}	66.0 (8.11)^{b}	2.0 (7.95)^{b}
T_{12}	Untreated Control	10.33 (3.21)^{bc}	291.0 (16.97)^{de}	301.33 (17.27)^{d}	13.33 (21.37)^{c}
	SEm±	0.19	0.63	0.62	1.29
	CD (P=0.05)	0.56	1.84	1.80	3.76

possibility of development of bitter taste in the food grains due to neem, they were not considered as suitable grain protectants. The presence of highly bioactive compounds such as asarone in sweet flag (Park et al., 2003), piperine in pepper (Scott et al., 2008) and eugenol in clove (Kafle and Shih, 2013) offer promise.

The present findings are in line with the earlier reports of insecticidal potential of clove (Aziz and Shadia, 2001), sweet flag (Sharma and Meshram, 2006) and pepper (Scott et al., 2008). Aslam et al. (2002) observed prominent growth inhibition of chickpea beetle with black pepper and clove powders. Likewise, Yusuf et al. (2011) and Subhadra and Kalita (2011) observed less fecundity and adult emergence of bruchids with minimum grain damage and weight loss in cowpea and green gram, respectively when treated with sweet flag. Kaur et al. (2019) reported that sweet flag powder resulted in 64.49,84.44 and 98.89 % mortality of *C. chinensis* at 5% after 1, 3 and 7 days of treatment, respectively. Abhijith et al. (2019) observed 100% mortality of cigarette beetle *Lasioderma serricorne* (L.) and rusty grain beetle *Cryptolestes ferrugineus* (Stephens) when exposed to sweet flag 6 EC treated package materials after 72 and 48 hr after treatment. The present results are in agreement with earlier ones on the efficacy of black pepper powder (Thakur and Mandeep, 2013; Islam et al., 2013; Poornasundari and Daniel, 2015).

Response under dual choice condition: The three spices, clove, black pepper and sweet flag were further evaluated under dual choice conditions. In all the cases, very few or less number of insects moved in to the containers having treated grain. However, black pepper (5.50) treatment received more number of adults compared to sweet flag (0.5) and clove (1.0) while the untreated controls received the mean number of adults ranging from 20.75 to 25.25 (Table 2). The adult emergence and grain damage at 40 and 80 days after release were also negligible in all the spice powder treatments However, slightly higher bruchid population

Table 2. Preference of *C. maculatus* to spice powder treated black gram- dual choice condition

Treatment	Insects moved into grain (No.)	Adult emergence (No.)		Grain damage (%) 80 DAR
		40 DAR	80 DAR	
Sweet flag @ 0.4%	0.50	2.0	4.50	1.0
Untreated	22.50	91.75	355.75	52.25
Black pepper @ 0.4%	5.50	12.25	29.0	4.25
Untreated	20.75	159.0	231.0	70.75
Clove @ 0.4%	1.0	1.0	1.50	0.50
Untreated	25.25	210.25	232.25	95.0

*Significantly differing- Student's *t*- test ($p < 0.05$).

and grain damage in black pepper treatment indicated that black pepper could not exert fumigant effect. Pea seeds were protected from pulse beetle damage up to 8 months with the treatment of essential oil of sweet flag at 2.5 ml/kg without deleterious effect on seed germination and vigour index (Kumar et al., 2018).

Spice powder tablets

Tablets prepared and placing them over the surface of grain led to negligible oviposition by the adults except black pepper alone (6.33 eggs) and untreated control (7.0 eggs) (Table 3). After 40 days after release of insects the adult emergence was nil or negligible in these treatments while there were 18.67 and 51.67 adults emerged from black pepper and untreated control respectively. Similar trend was observed in adult emergence at 80 and 120 days also; in 120 days, tablets made of sweet flag and clove powders alone or in combination with clove oil could result nil or negligible adult emergence. The grain damage was also negligible in the sweet flag and clove powder tablet treatments. Higher number of adults emerged from pepper tablet treatment also confirmed that it failed to exert insecticidal effect on pulse bruchid. On the other hand, negligible adult emergence and grain damage recorded with pepper tablet impregnated with clove oil further indicated that the effect was only due to fumigant toxicity of clove oil.

Table 3. Efficacy of spice powder tablets against *C. maculatus*

Tr. No.	Treatment	No. of eggs at 10 DAR	Adult emergence (No.)				Grain damage (%) at 120 DAR
			40 DAR	80 DAR	120 DAR	Total	
T_1	Sweet flag (1 g)	1.67 (1.46)[a]	0.0 (0.70)[a]	0.0 (0.70)[a]	0.0 (0.70)[a]	0.0 (0.70)[a]	0.0 (0.62)[a]
T_2	Black pepper (1 g)	6.33 (2.46)[b]	18.67 (4.35)[b]	243.67 (14.75)[b]	188.67 (13.44)[c]	451.0 (21.19)[b]	93.0 (74.68)[b]
T_3	Clove (1 g)	1.33 (1.35)[a]	0.33 (0.88)[a]	0.67 (1.05)[a]	0.0 (0.70)[a]	1.0 (1.18)[a]	0.67 (4.04)[a]
T_4	Sweet flag (1 g) + Clove oil 200 µl	0.0 (0.70)[a]	0.0 (0.70)[a]	0.33 (0.88)[a]	0.0 (0.70)[a]	0.33 (0.88)[a]	0.33 (2.33)[a]
T_5	Black pepper (1 g) + Clove oil 200 µl	1.33 (1.29)[a]	0.67 (0.99)[a]	1.0 (1.18)[a]	0.0 (0.70)[a]	1.67 (1.38)[a]	0.67 (4.04)[a]
T_6	Clove (1 g) + Clove oil 200 µl	0.67 (1.05)[a]	0.0 (0.70)[a]	0.0 (0.70)[a]	0.0 (0.70)[a]	0.0 (0.70)[a]	0.0 (0.62)[a]
T_7	Untreated Control	7.0 (2.68)[b]	51.67 (7.18)[c]	462.33 (21.51)[c]	68.33 (8.22)[b]	582.33 (24.14)[c]	95.0 (77.50)[b]
	SEm±	0.32	0.27	1.39	0.82	0.48	1.46
	CD (P=0.05)	0.96	0.84	4.21	2.50	1.46	4.43

Values in parentheses transformed values; DAR: Days after release

Sharma and Meshram (2006) observed no infestation of *S. oryzae* in stored wheat with application of essential oils of sweet flag and clove at 500 ppm. Similarly, Kheradmand et al. (2015) observed the repellent effect of clove essential oil against spider mite. Clove oil is known for its oviposition inhibition, repellent activity and insecticidal effects on several stored pests. The essential oil of clove caused 100% mortality of bean weevil and maize weevil at the concentration of 35 µl/g (Carlos et al., 2016) and cowpea beetle at 0.25% concentration after 48 h of treatment (Abdullah et al., 2017). Keeping a plywood piece impregnated with clove oil @ 0.5 ml on grain surface offered better protection of green gram from pulse bruchids (Swamy and Sandeep, 2018).

Thus, sweet flag, clove and pepper powders were effective for suppression of *C. maculatus* as grain mixing at 0.4% in black gram. In dual choice tests clove and sweet flag showed repellent effect with fumigant activity. The spice tablets made of sweet flag and clove powders alone or fortified with clove oil when placed on the grain surface offered grain protection from pulse bruchid with very negligible oviposition and no adult emergence.

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