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Control of Cowpea Weevil (*Callosobruchus maculatus* L.) in Stored Cowpea (*Vigna unguiculatus* L.) Grains using Botanicals

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Abstract: An investigation to assess the effectiveness of locally available botanicals in the control of cowpea weevil (*Callosobruchus maculatus* L.) was done in the Department of Crop Science and Production laboratory of Sokoine University of Agriculture Morogoro, Tanzania. The trial was conducted during September 2004-January 2005. The test materials consisted of two commercial cultivars of cowpea namely Fahari and Tumaini; 8 botanical protectants and their combinations and two controls viz., no protectant and standard actellic super dust, making a total of 13 treatments. A split plot experiment in which the two commercial cultivars were the main plots while the 13 protectant treatments comprised the subplots was used as a Randomized Complete Design with four replications. Cultured pest weevils were placed in vials containing cowpea seeds and the respective sub-plot treatments including the two controls; ashes of rice husks, kitchen and cow dung; powders of dried leaves of *Tephrosia vogelii*, neem, pyrethrum flowers, tobacco snuff, black pepper fruit and their various combinations. Data were recorded after 10 weeks of storage on number of bruchids after treatment, number of holes per seed, numbers of damaged and undamaged seeds. Protectants had varying degrees of effectiveness in controlling the cowpea weevil. Leaf powders of *Tephrosia vogelii*, tobacco snuff, actellic super dust and combination of leaf powders of neem and *Tephrosia vogelii* significantly excelled the control of no protectant in controlling the cowpea weevil. Black pepper powder gave significantly better results than the control in suppressing bruchid survival, higher numbers of undamaged seeds and fewer holes per seed. The cowpea cultivars did not differ significantly on the studied traits. Leaf powders of *Tephrosia vogelii*, tobacco snuff and combination of neem and *Tephrosia* are effective in controlling stored grain of cowpeas. Black pepper powder can as well be used for cowpea grain storage particularly where the aforementioned botanicals are not available.

Key words: Actellic super, black pepper, *Tephrosia*, tobacco snuff

INTRODUCTION

Cowpea (*Vigna unguiculata* L.) is a pulse crop that can be grown successfully in extreme environments such as high temperatures, low rainfall and poor soils with a few inputs[1]. Subsistence farmers in the semi-arid and sub-humid regions of Africa are the major producers and consumers of cowpea[2,3]. Cowpea grain is important to the incomes of resource poor farmers as well as to the nutritional status and diets of people in West and East Africa, Latin America and the Carribean basin[3]. The seed is high in protein contents and can be consumed directly, make flour, sprouts, weaning foods for young children and thus ameliorating malnourishment, wasting and stunting[2]. It also provides a useful complement in diets comprised mainly of roots, tubers or cereals. However, post harvest losses of cowpea grain are a serious problem, and in Africa, as much as 20-50% of grain is lost because of infestations from the pest[4]. Infestation results into weight loss and quality deterioration. Heat, moisture and waste products produced by the weevil also result into further deterioration and the growth of moulds[5]. This

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renders cowpea grain unfit for consumption and selling. Thus, farmers are forced to sell their products early after harvest when prices are still low partly because of anticipated losses in storage.

In the past, infestation was often a less serious problem because farmers cultivated traditional varieties which though low yielding, were generally more resistant to attack by pests. However, the introduction of high yielding pulse varieties has resulted in increased storage losses since they are usually more susceptible to pest damage[5]. The pesticidal properties of many plants have been known for a long time and natural pesticides based on plant extracts such as rotenone, nicotine and pyrethrum have been commonly used in pest control during the earlier half of this century[6]. However, after the world war II, they lost their importance with the introduction of synthetic organic chemicals, which were concentrated products with a high knock down effect on pest organisms. The use of these organochlorines and organophosphorus compounds however, has led to the hazardous effects on environment and human beings[7]. In addition, synthetic pesticides are not easily available among poor resource farmers, and when available, are too expensive[8-10]. As such, farmers have reverted to the usage of natural products that include plant extracts, powders, ashes, minerals, cow dung, oils, and sand in the control of pests with varying levels of effectiveness[11-13]. Botanicals have been found to have broad spectrum insecticidal properties with reduced persistence and toxicity in relation to organochlorines and organophosphorus compounds. They are easily available, can be produced within farmers' vicinity providing a more sustainable approach to pest control[7,14]. For instance, cowpea seeds treated with bark and fruit powders from Mazedarch, black pepper (*Piper nigrum*) fruits, leaves of Eucalyptus, *Croton gratissilum*, *Spirostychus africana*, *Ochna pulera*, clove flowers and *Solanum nigrum* were better protected than untreated seeds[7]. Thus, more investigations are necessary to explore natural protectants available in the local areas for a more sustainable approach in controlling storage pests.

The present investigation was aimed at assessing and identifying alternative and effective botanicals available within the farmers' environment that can be recommended as alternative low cost technique of minimizing post-harvest losses of cowpea grain from the cowpea weevil.

MATERIALS AND METHODS

The experiment was conducted in the laboratory of the Department of Crop Science and Production, Sokoine University of Agriculture, Morogoro, Tanzania during September, 2004 to January, 2005. Seeds of two commercial cultivars of cowpea viz., Tumaini and Fahari were obtained from the Bean Improvement Unit of the Sokoine University of Agriculture, Morogoro, Tanzania. Storage pests (cowpea weevils) were isolated from infested seeds collected from the Morogoro municipal market and the insects were placed into a bottle containing undamaged cowpea seeds for culturing of the pest during September, 2003. The contents were kept in an incubator maintained at 28°C and 75% RH for 60 days in order to get insect weevils for use in the study. One kilogram of seeds for each variety was placed in a refrigerator for two weeks so as to sterilize the seeds.

Various botanicals and their combinations were used for controlling the weevils. A split plot arrangement in a Randomized Complete Design was used for arrangement of treatments. The two varieties of cowpeas were the main plot treatments while the various botanicals and their combinations were the sub-plot treatments. The subplot treatments consisted of the following:

T_0 : Control: no protectant
T_1 : 2% (w/w) cow dung ash
T_2 : 2% (w/w) *Tephrosia vogelii* leaf powder
T_3 : 2% (w/w) pyrethrum flower powder
T_4 : 3% (w/w) tobacco leaf powder snuff[15]
T_5 : 2% (w/w) rice husk ash
T_6 : 2% (w/w) Black pepper fruit powder[16]
T_7 : 5% (w/w) neem leaves powder[17]
T_8 : 2% (w/w) kitchen ash
T_9 : Combination of 1% neem leaves and 1% *Tephrosia vogelii* (w/w)
T_{10} : Combination of 1% cow dung ash and 1% kitchen ash
T_{11} : Combination of 1% cow dung ash and 1% pyrethrum powder
T_{12} : Control: 1% standard actellic supper dust.

Leaves and flowers of the respective botanicals were dried in shade with diffuse light and then ground into fine powder using a milling machine. One hundred and four glass vials, each containing 25 seeds of each of the two varieties and 13 treatments in 4 replications were used for the study. For each of 104 vials, seven pairs of newly emerged cowpea weevils were added. Data were collected in each vial at 10 weeks after infestation on number of damaged and un-damaged seeds, number of live bruchids and number of holes on seeds. The following variables were derived from collected data:

a) % damaged seeds was obtained by dividing the number of damaged seeds by total number of seeds and multiplied by 100.
b) Number of bruchids was obtained by counting live bruchids in each vial at the end of the tenth week and recorded.

c) Average number of holes per seed was obtained by dividing number of holes in all seeds in a vial by number of seeds.
d) Number of damaged seeds was obtained by counting all the seeds in each vial with at least one hole from bruchid infestation.
e) Number of undamaged seeds was determined by counting all the seeds in each vial that contain no hole from bruchid infestation.

The variables were subjected to standard statistical analysis using the following statistical model:

$$X_{ij} = u + V_i + E_{(a)} + T_j + VT_{(ij)} + E_{(b)}$$

Where:

X_{ij} = An observation in the ith variety at the jth treatment
u = The general effect
V_i = The i^{th} varietal effect
$E_{(a)}$ = main plot error
T_j = The j^{th} treatment effect
$VT_{(ij)}$ = The interaction between ith variety and jth treatment
$E_{(b)}$ = Experimental error

RESULTS

The various botanical treatments differed significantly in all the variables viz., number of bruchids after infestation, number of damaged seeds, number of un-damaged seeds, average number of holes per seed and percent damaged seeds. There was no significant variety or variety x treatment interaction effects in any of the variables (Table 1).

Mean effects of botanical treatments and relationships among variables

% damaged seeds: The lowest percentage of damaged seeds was obtained with tobacco snuff (T_4) (0.5%) and actelic super dust (T_{12}) (3%) (Table 2). These were followed by *Tephrosia vogelii* (T_2) (7%), combination of neem and *T. vogelii* powders (T_9) (16%) all of which performed significantly better than the control of no protectant (T_0) in having low percentages of damaged seeds. On the other hand, the highest percent damage (98.5%) was observed with pyrethrum powder (T_3) followed by kitchen ash (T_8) (94%). The control treatment where there was no application of any protectant (T_0), had 33.1% damaged seeds.

Number of bruchids after treatment: The lowest number of bruchids after treatment was observed on tobacco powder (T_4) (7.2) and *Tephrosia vogelli* (T_2) (9.4). These were followed by actellic super dust (T_{12}) (11.5), combination of neem and *T. vogelii* (T_9) (14.8) and black pepper powder (T_6) (20.9). Cow dung ash (T_1) also excelled significantly the control of no protectant in having low number of bruchids. The highest number of bruchids (60.2) was observed on pyrethrum powder (T_3), followed by kitchen ash (T_8) (56.6). The no protectant control treatment (T_0) had 31.4 bruchids per vial (Table 2).

Number of holes per seed: The lowest average number of holes per seed (0.1) was obtained with tobacco snuff (T_4) and this was the only treatment that was significantly better than the control of no protectant application on this variable. On the other hand, the highest number of holes per seed was obtained in pyrethrum treatment (T_3) (5.6) while the no protectant control treatment (T_0) had 2.6 holes per seed (Table 2).

Number of damaged seeds: The lowest number of damaged seed was obtained with tobacco snuff (T_4) (0.1) followed by *Tephrosia vogelii* (T_2) (1.8) and actellic super dust (T_{12}) (2.1). These were followed by combination of neem and *T. vogelii* (T_9) (4.0) and all of these were statistically the same and significantly better than the control of no protectant application. The highest number of damaged seed was obtained with pyrethrum (T_3) (24.6) and kitchen ash (T_8) (23.5) while the no protectant control treatment had 9.1 damaged seeds (Table 2).

Number of undamaged seeds: The highest number of undamaged seeds was obtained with tobacco snuff powder (T_4) (24.3) followed by *Tephrosia vogelii* (T_2) (23.9), actellic super dust (T_{12}) (22.4) and combination of neem and *T. vogelii* (T_9) (20.8). Other treatments that

Table 1: ANOVA summary for the studied variables

SOV	df	No. of bruchids	No. of damaged seeds	No. of undamaged seeds	No. of holes per seed	% damaged seed
Variety	1	39.4	180.5	332.7	14.6	3313.1
Error	6	555.7	108.1	127.8	2.6	1704.3
Treatments	12	2299.9**	516.9***	526.3***	15.2*	8650.7***
Var x Treat	12	542.1	57.5	65.1	6.2	888.4
Error	64	337.0	50.6	46.5	6.9	870.5
Total	95	3774.1	913.6	1098.4	45.5	15427.0

* $p=\leq 0.05$, ** $p=\leq 0.01$, *** $p=\leq 0.001$

Table 2: Mean effects of botanical treatments for the studied variables

Treatments	No. of damaged seeds	No. of undamaged seeds	No. of holes per seed	% damage	No. of bruchids
Cow dung ashes	9.1	15.9	2.1	36.5	28.4
Tephrosia vogelii	1.8	23.9	0.6	7.0	9.4
Pyrethrum powder	24.6	0.4	5.6	98.5	60.2
Tobacco snuff	0.1	24.3	0.1	0.5	7.2
Rice husks ashes	14.5	11.5	2.3	58.0	31.9
Black pepper powder	10.9	15.6	1.4	43.5	20.9
Neem	19.6	5.4	1.5	78.5	43.9
Kitchen ashes	23.5	1.5	2.6	94.0	56.6
Neem + *T. vogelii*	4.0	20.8	1.1	16.0	14.8
Cow dung+Kitchen ashes	13.8	11.3	1.6	55.0	33.3
Cow dung + Pyrethrum powder	13.5	11.5	1.4	54.0	34.1
Actellic Super Dust	2.1	22.4	0.6	3.0	11.5
Control	12.1	8.0	2.6	33.1	31.4
x	11.5	13.2	1.2	44.4	29.5
SEx (+/-)	2.5	2.4	0.9	10.4	6.4
$LSD_{0.05}$	5.9	5.7	2.2	24.6	15.3
CV (%)	61.8	51.7	218.9	66.4	62.2

Table 3: Phenotypic correlations among the variables

Variable	1	2	3	4
No. of bruchids after treatment	-	0.7***	-0.8***	0.3*
No. of damaged seeds		-	-0.8***	0.4*
No. of undamaged seeds			-	-0.5**
No. of holes per seed				-

* $p \le 0.05$, ** $p \le 0.01$, *** $p \le 0.001$

performed significantly better than the no protectant control in having high number of undamaged seeds were rice husk ash (T_5), black pepper powder (T_6), cow dung ash (T_1), combination of cow dung and pyrethrum powder (T_{11}) and actellic super dust (T_{12}). The lowest number of undamaged seeds was obtained with pyrethrum powder (T_3) (0.4) and kitchen ash (T_8) (1.5).

Tephrosia vogelii (T_2), tobacco snuff powder (T_4), combination of neem and *T. vogelii* (T_9) and actellic super dust (T_{12}) performed significantly better than the no protectant control in all of the variables. These were followed by black pepper powder (T_6) that was significantly better than the no protectant control (T_0) in three variables viz., numbers of bruchids, holes per seed and undamaged seeds (Table 2).

Phenotypic correlations among the variables: Significant positive relationships were observed among number of bruchids after treatment, average number of holes per seed and number of damaged seeds (Table 3). On the contrary, significant negative relationships were observed between number of undamaged seeds with number of bruchids after treatment and number of damaged seeds; average number of holes per seed with number of undamaged seeds.

DISCUSSION

The positive relationships observed among bruchids and levels of seed damage and negative relationships between bruchids and undamaged seeds confirm that higher bruchid populations result to higher levels of stored grain damage, necessitating for a need to control pest populations to low or zero levels in storage. The present investigation indicates that farmers can use local species of plants for protecting cowpea grains against the cowpea weevil after harvest. Thus, for example, tobacco snuff, *Tephrosia vogelii* powder, combination of neem and *Tephrosia vogelii* gave significantly better control of the pest than the control where no pesticide was applied. Similarly, actellic super dust also showed superiority over the control in all the variables investigated. Powdered leaves of *Tephrosia* (vern. "utupa") have been found to have the suffocation effect and contains an ingredient that has a direct insecticidal and larvicidal effect[18]. In Nigeria, *Tephrosia vogelii* has been found effective against pests of stored grain legumes and cereals particularly against *Zabrotes subfasciatus* and *Acanthoscelides obtectus*[18]. Powder of *Tephrosia* leaves has also been found to have pesticidal effects in the field[6] and in storage. In Congo for example, *Tephrosia* powder mixed with groundnut at a ratio of 1:40 gave a 98.8% mortality of the groundnut borer, *Coryedon serratus* after 13 days of storage[12]. It is therefore suggested that *Tephrosia* is a broad spectrum botanical pesticide that can be used for the control of both field and storage pests. The good control for the cowpea weevil observed from the combination of neem and *T. vogelii* suggests that the control is predominantly due to the effect of *Tephrosia* rather than that of neem. This is because neem on its own, gave rather poor control while *Tephrosia* gave good control of the storage pest. However, in the event that higher levels of *Tephrosia* may pose hazard or ill effects to consumers, combination of neem with *Tephrosia* may help to reduce the concentration of dust formulation of *Tephrosia* per unit weight of grain protected. Neem dust may be used as a carrier of *Tephrosia* dust since the neem leaves also have some pesticidal effects to a lesser extent (Table 2). In the present study, neem leaf powder was not significantly effective in controlling the weevil. However, other studies using neem oil and kernel powder have proved effective as storage protectants against cowpea weevil, bean bruchid, beetles and grain borers[12,19]. Thus different plant parts can give different effects on the storage pest. Neem leaves have been found to contain much less of the major active ingredient, azadirachtin, than seed kernels and therefore, not commonly used[7,13]. The present study indicated the good performance of actellic super dust in controlling the storage pest. Actellic super is a commercially recommended insecticide against a wide range variety of storage pests of cereals and grain legumes. However, the availability and costs associated with synthetic pesticides are well known, rendering the usage of actellic super dust among poor resource farmers prohibitive. *Tephrosia* is a potential source of rotenone, an important non residual insecticide[20]. The plant has as well been found to be effective against a number of hard-to-kill field insects including cucumber beetle, harloquin bug, squash bugs, thrips, scales, mites, leaf hoppers, flea beetles, spittle bugs and some fruit worms[21]. However rotenone, an insect stomach poison, has largely been used to control field pests rather than storage pests. The pesticide is toxic to fish, shrimps and crabs and highly irritating to the human user, necessitating the use of respirators and protective clothing during field application. Rotenone has been found to have little risk to people and other animals because it decomposes easily in heat and hence upon cooking and in the digestive tract[22]. Thus it seems to be a good pesticide against storage pests with little possibility of having harmful effects to consumers since also, consumers always wash grain before use or milling, as it is with other commercial pesticides. *Tephrosia vogelii* therefore stands to be an effective and relatively safe storage pesticide. Safer application methods and the solubility in water of *Tephrosia* dust needs to be well established since this will determine efficient removal of the dust upon washing.

The use of tobacco powder as a potent storage pesticide has as well been demonstrated by other workers in controlling the cowpea weevil[15,23]. Grain weevils are amongst the target organisms of tobacco botanical pesticide[24,25]. According to Elwell and Maas [26], tobacco is a broad spectrum poison, with mechanisms of control being a stomach and a respiratory poison, insecticidal and repellant. Constituents of tobacco include nicotine, nornicotine and anabasine[27]. Nicotine, an alkaloid, is believed to be an active ingredient against insect pests although it is highly toxic to mammals. Poisoning in human beings and livestock has been reported from intestinal or accidental misuse of nicotine products. According to Duke[28], the alkaloid is readily absorbed after either ingestion, inhalation or through skin and it is fatal in small amounts. Despite that nicotine is highly toxic to mammals; it continues to be used against various insect pests[6,29]. The use of poisonous dust formulations in controlling storage pests is common, for instance the widespread usage of actellic super dust. However, care is exercised in handling of the formulation during application and through washing of grain before use or milling. It should however be noted that the tobacco dust used in the present investigation is a snuff that is locally prepared for snuffing. Preparation of the snuff includes the use of tobacco leaves, "magadi" ($CaCO_3$) and such a snuff does not seem to have apparent harmful effects to users. The local people have for a long time been using snuff without apparent ill effects. Further research is necessary however, to investigate the levels of risk and safety of tobacco snuff as compared to normal leaf powder and other storage pesticides. Studies need to be conducted on the safety of tobacco powder treated grains after washing out with water and subsequent drying in sunlight or other sources of heat. Similarly, investigations need to be conducted to ascertain the effect of moisture in tobacco snuff with respect to residual effect on and within grains. If the residues are apparently absorbed within grains, then the use of tobacco snuff should only be used for protecting cowpea grain as seed rather than for consumption purposes. However, this will be possible if research findings indicate that there is no significant detrimental effect of tobacco snuff on germination capacity. Research is also needed to find out the difference in safety and effectiveness between tobacco snuff and the sole leaf powder as storage pesticides.

Black pepper also had substantial effects in controlling the cowpea weevil particularly when considering their significantly better control with respect to surviving bruchids, number of undamaged seeds and holes per seed. Other workers have also reported that Piper is among the most promising candidate plant materials for consideration as future grain protectants[20]. Black pepper was able to kill more than 90% of cowpea weevil within 48 h after the contact[30]. Similarly, cowpea seeds treated with fruit powder of black pepper were better protected than untreated seeds against cowpea weevils in an experiment involving 12 botanicals[4,7,26]. Su[31] reported that oil of black pepper extracted in hexane reduced F1 emergence of cowpea weevil on cowpea. Fruit powder of *Piper guineense*, was found to have comparable best effects as actellic super dust on fecundity of the cowpea weevil in a trial involving 3 botanicals and the synthetic pesticide[32]. These findings are consistent with results of this study where black pepper powder reduced significantly the number of bruchids and consequently the number of damaged seeds. Black pepper is a spice used in food seasoning and therefore it does not pose a health hazard either to consumers or the environment. In areas where black pepper thrives well, it is advised that farmers grow the crop not only for commercial use and as spice but also as a good protectant of products against the cowpea weevil. Constituents of many aromatic plants used for flavoring have been found to possess insecticidal properties[33].

The current investigation has revealed the potential of botanicals in controlling cowpea weevil in storage of cowpea grains. Leaf powders of *Tephrosia*; combination of neem and *Tephrosia* and tobacco snuff significantly excelled the control in their effectiveness and thus should be used for storing both food and seed grains. Similarly, black pepper powder may as well be used for controlling the storage pest since they pose minimal risk to consumers and the environment. In as much as the synthetic pesticide viz., actellic super dust was significantly superior to the control and excelled many botanicals, it should be used with care, taking into consideration availability, expenses, environmental and health hazards during and after application.

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