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Activities of Rosemarinus officinalis powders against three adult storage pests of Zea mays (L.), Vigna unguiculata (L.), and Sorghum vulgare (L.)

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Abstract
Three storage pests Sitophilus zaemais, Callosobruchus maculatus and Tribolium castaneum were separately subjected to direct contact with powders of Romarinus officinalis and permethrin was used as a positive control in the bioassay. The insecticidal activity of the powder on each of the pests was determined by its toxicity. At 2.50 (wt/wt), of R. officinalis on S. zeamais highest toxicity was recorded at all periods of observations with no significant differences. Similarly, the results of activities of R. officinalis on C. maculatus at all doses significantly (P≤0.001) controlled adult C. maculatus when compared with the control. The treatment dose 0.25 (wt/wt) of R. officinalis on adult T. castaneum (P≤0.001) significantly effected control at all periods of observations. Regardless of the pest under study, R. officinalis at 2.50 (wt/wt) was significantly the most effective when compared with other doses in the bioassay except when compared with the level of toxicity of permethrin to any of the beetles under investigation.

Keywords: *Rosemarinus officinalis, Sitophilus zeamais, Callosobruchus maculatus, Tribolium castaneum, Toxicity, Powder and adult pests.*

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I. Introduction

Initial infestation of food crops like cowpea, groundnut, maize, rice, sorghum, wheat often occur in the field just before harvest and are carried into the store where the population builds up rapidly (Deshpande *et al*., 2011). Nigeria farmers harvest their yields with pod or the plant as a whole often heaped and abandoned until such is needed (Bawa *et al*., 2012). This provides a dangerous form of inoculum for store infestations during a storage period that may be as long as 9 months in many parts of Nigeria (Ogunkoya and Dawodu, 2014). The severity of damage caused by these pests lowers the quality and quantity of these crops where available for consumption as 100% destruction of unprotected pulses and grains in storage within 9 months have been reported (Alao and Adebayo., 2011; Bawa *et al*., 2012; Babarinde *et al*., 2016). The protection of crops such that ensure sustainability of the value-chain has been largely achieved by the use of synthetic chemicals (Dahiru *et al*., 2014). Dawodu *et al*., (2021b) reiterated that the intensive use of these chemicals violate ecological principles leading to serious problems such as pest resistance, increase in cost of pest control and persistence of dangerous chemical compounds in the environment. Persistence use of synthetic insecticides results in negative effect on non-target organisms, un-biodegradability and bio-magnifications of pesticide residue through food chains (FAO/WHO, 1985). Similarly, series of queries have been raised from suspicions of reproductive health related diseases and other cholinergic syndrome in man which have been traced to poison from pesticides (PAN, 2012; Abdullazadeh *et al* 2015). These poison has found its way into man via skin, oral ingestion and through nose inhalation (Bjorling-Poulsen *et al*., 2012; Ojo *et al*., 2013; Sharifzadeh *et al*., 2017). For these reasons alternative strategies needed be develped in order to avoid those calamities associated with the continuous use of synthetic chemicals.

The importance for the search for alternative to the use of synthetic insecticides cannot be over emphasized. Plants with insecticidal properties have over the years proved to be useful as alternative to synthetic insecticides in the protection of stored products from the damage and destruction caused by storage pests. A greater number of insecticidal plants powders (*Azadirachta indica* and *Piper guineense*) have been recommended for use without dilution of the active ingredients (Olaifa and Erhun, 1988; Olayinka- Olagunju, 2014; Okirikata *et al*., 2015). One of such is when powder formulations in its solid active ingredient is applied in the dry form in order to control storage pest (Ekeh, *et al*., 2013; Dawodu *et al*., 2022). The application of dust does not require a solvent and the higher the dose the greater its toxicity on the receptor pest (Ofuya and

Osadahun, 2005). Although with high relative humidity in the storage, efficacy is reduced (Mahdi and Khalequazzaman, 2006). Application of powders with insecticidal properties on stored products can be done by the use of motorized knapsack prayers, it could be sprinkled around sacks or blown into crevices by hand (Olotuah *et al*., 2013). The toxicant effect of these powders on stored pests may come in form of repellants, affect egg lay, could be antifeedant, distortion in developmental growth, causes respiratory disorder or insect pest mortality (Ofuya and Dawodu 2002; Isman, 2017). One of the many plants with great insecticidal potential *R. offinalis* has been found to help in the control of beetle pest of rice *S. oryzae* (Lampiri *et al*., 2020). There is dearth in the search for its potentials on *S. zeamais*, *C. maculatus* and *T. castaneum*. Therefore, the main reason for this study is to identify the potentials of *R. offinalis* in the control of stored pests affecting major crops in Nigeria.

II. Materials And Methods

Site

The experiment was carried out in the Agronomy laboratory of the Department of Agricultural Education, College of Education Ikere-Ekiti, Nigeria. The botanical materials used was obtained from the Forestry Research Institute of Nigeria (FRIN), Ibadan. Authentication of the plant material was subsequently made at the herbarium of the institute but voucher numbers were not documented in this research for propriety reasons.

Beetle culture

Cultures for each insect was separately collected from the existing colonies which are maintained in Kliner jars for over 30 generations in the laboratory of College of Education, Ikere-Ekiti. Each adult beetle was reared at ambient room temperature $30 \pm 2\ ^{0}c$ and relative humidity of between 68 and 80% throughout the period of the bioassay according to procedures described by Babarinde *et al.* (2011). Contact toxicity was employed to assess and determine the potentials of the developed botanical product against *S. zeamais*, *C. maculatus* and *T. castaneum*. The powder based experiment consisted of a total of 12 treatments which was replicated 5 times. Each experiment consisted of positive and negative controls. The duration of potency of each botanical formulation was determined within a stipulated period of 10 months.

Data collected was subjected to analysis of variance (ANOVA) using statistical analysis system (SAS) version 9.3 software and significant means was compared at 5 % significant level using Least Significant Difference (LSD).

III. Results

Rosmarinus officinalis leaf powder produced significant toxic effect against adult *S. zeamais* ($P \leq 0.001$). Permethrin produced significantly higher toxicity than the doses of *R. officinalis* and the control treatment at all periods of observation (Table 1). 2.5% wt./wt. of *R. officinalis* produced the significantly highest toxic effect out of the doses at all period of observation (22.48, 39.26, 52.28, 55.40, 63.84 and 80.20) which was mostly not significantly different from the toxicity produced by 2.25% wt./wt. while 0.25% wt./wt. produced the lowest toxic effect that was significantly higher than the toxicity produced by the control treatment at all periods of observation except at 24 HAT (Table 1).

Table 1: Toxicity of *Rosmarinus officinalis* leaf powder against adult *Sitophilus zeamais*

Dose (wt/wt)	Days After Treatment					
	24 HAT	**48 HAT**	**72 HAT**	**96 HAT**	**120 HAT**	**144 HAT**
0.25%	0.00±0.00[a]	24.40±3.48[b]	30.98±2.89[cd]	38.32±3.63[c]	42.38±2.14[bcd]	44.18±2.70[b]
0.50%	13.92±5.85[b]	25.17±2.44[b]	38.84±1.75[d]	42.34±1.51[c]	45.46±3.06[cd]	49.42±2.26[bc]
0.75%	14.37±3.60[b]	29.84±5.57[b]	30.04±2.71[cd]	33.60±1.89[bc]	40.96±1.95[bcd]	48.84±1.24[bc]
1.0%	15.80±4.78[b]	23.75±2.15[b]	30.20±2.82[cd]	33.48±2.42[bc]	36.90±3.73[bc]	51.46±1.43[bcd]
1.25%	15.78±4.70[b]	26.68±5.84[b]	13.92±5.85[b]	27.16±2.69[b]	35.80±1.78[b]	53.98±1.90[cde]
1.50%	22.60±1.79[b]	20.90±6.15[b]	25.92±3.83[c]	34.42±2.98[bc]	44.96±1.40[cd]	57.16±0.14[de]
1.75%	16.04±4.59[b]	26.34±7.32[b]	34.96±2.82[cd]	38.08±1.05[c]	50.00±0.81[de]	59.56±2.53[e]
2.0%	21.45±4.02[b]	21.22±6.43[b]	36.92±1.56[cd]	38.64±1.48[c]	55.72±3.65[ef]	68.32±2.00[f]
2.25	22.26±1.81[b]	33.38±2.75[b]	47.64±2.46[e]	50.12±1.95[d]	58.24±2.37[fg]	70.58±1.89[f]
2.50%	22.48±3.49[b]	39.26±2.77[b]	52.28±3.33[e]	55.40±2.65[d]	63.84±2.26[g]	80.20±4.04[g]
Permethrin	90.00±0.00[c]	90.00±0.00[c]	90.00±0.00[f]	90.00±0.00[e]	90.00±0.00[h]	90.00±0.00[h]
Control	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
ANOVA	df= 11,59	df= 11,59	df= 11,59	df= 11,59	df= 11,59	df= 11,59
Result	F= 42.811	F= 22.338	F= 57.540	F= 93.329	F= 85.659	F= 120.148
	P≤ 0.001	P≤ 0.001	P≤ 0.001	P≤ 0.001	P≤ 0.001	P≤ 0.001

*Values are means±SE

*Means followed by the same letter of alphabet within the column are not significantly different using SNK at 5% probability level

Rosmarinus officinalis leaf powder similarly produced significant toxic effect against adult *Callosobruchus maculatus* ($P \leq 0.001$). Permethrin produced significantly higher toxicity than the other doses of *R. officinalis* and the control treatment at all periods of observation. There were however no significant difference among the toxicity produced by the other doses of *R. officinalis* which were significantly higher than the toxicity observed from the control treatment between 24 and 144 HAT (Table 2).

Table 2: Toxicity of *Rosmarinus officinalis* leaf powder against adult *Callosobruchus maculatus*

	Days After Treatment					
	24 HAT	**48 HAT**	**72 HAT**	**96 HAT**	**120 HAT**	**144 HAT**
0.25%	20.96±5.74^{bcd}	29.24±1.61^{b}	35.96±2.29^{b}	37.16±2.24^{b}	38.38±2.14^{b}	51.06±2.13^{bc}
0.50%	23.32±2.00^{bcd}	23.32±2.00^{b}	30.32±3.92^{b}	35.24±5.34^{b}	36.88±4.17^{b}	45.20±4.62^{b}
0.75%	31.60±3.58^{d}	29.24±1.61^{b}	31.90±3.49^{b}	35.86±4.64^{b}	39.92±3.50^{b}	48.58±4.68^{bc}
1.0%	27.92±1.32^{cd}	30.44±2.53^{b}	37.56±2.10^{b}	46.14±1.53^{b}	45.34±2.17^{bc}	54.48±4.67^{bc}
1.25%	18.00±4.85^{bcd}	30.12±3.54^{b}	33.48±2.03^{b}	38.88±2.14^{b}	38.88±2.14^{b}	49.20±3.83^{bc}
1.50%	12.68±1.5.38^{abc}	30.12±3.54^{b}	31.96±2.88^{b}	36.88±4.17^{b}	36.88±4.17^{b}	43.62±3.77^{b}
1.75%	16.36±4.38^{bcd}	27.92±1.32^{b}	31.96±3.49^{b}	35.86±4.64^{b}	38.60±2.57^{b}	46.78±3.91^{b}
2.0%	9.00±5.66^{ab}	26.28±2.34^{b}	37.96±2.21^{b}	44.94±3.22^{b}	47.56±2.93^{bc}	45.00±3.66^{b}
2.25%	16.56±4.41^{bcd}	23.20±2.93^{b}	31.76±2.37^{b}	36.80±4.33^{b}	48.72±2.90^{bc}	56.10±4.69^{bc}
2.50g	21.98±2.20^{bcd}	26.36±3.54^{b}	38.62±3.40^{b}	46.52±507^{b}	55.7±2.98^{bc}	64.52±6.58^{c}
Permethrin	90.00±0.00^{e}	90.00±0.00^{c}	90.00±0.00^{c}	90.00±0.00C	90.00±0.00^{c}	90.00±0.00^{d}
Control	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
ANOVA	df= 11,59	df= 11,59	df= 11,59	df= 11,59	df= 11,59	df= 11,59
Result	F= 33.482	F= 72.782	F= 56.254	F= 29.993	F= 48.277	F= 25.281
	P< 0.001	P< 0.001	P≤ 0.001	P≤ 0.001	P≤ 0.001	P≤ 0.001

*Values are means±SE

*Means followed by the same letter of alphabet within the column are not significantly different using SNK at 5% probability level

Rosmaninus officinalis leaf powder produced significant toxic effect against adult *T. castaneum* ($P \leq 0.001$). Permethrin produced the highest observed toxicity across the period of observation which was significantly higher than the toxicity produced by all the doses of *R. officinalis* (Table 3). There were no significant differences among the doses of *R. officinalis* while 0.25% wt./wt. produced the lowest toxicity at these periods which was not significantly different from the toxicity of the control treatment between 24 and 72 HAT (Table 3). In all, dose 2.50% wt /wt recorded a progressive highest increase in toxicity with increase in hours after treatment between 24 and 72 hours when compared with other doses in the experiment (Table 3).

Table 3: Toxicity of *Rosmaninus officinalis* leaf powder against adult *Tribolium castaneum*

Dose (wt/wt)	**Days After Treatment**					
	24 HAT	**48 HAT**	**72 HAT**	**96 HAT**	**120 HAT**	**144 HAT**
0.25%	0.00±0.00^{a}	7.36±4.50^{ab}	7.36±4.50^{ab}	20.04±1.64^{b}	20.34±1.94	20.34±1.94^{b}
0.50%	0.00±0.00^{a}	7.36±4.50^{ab}	7.36±4.50^{ab}	20.04±1.64^{b}	23.32±2.00^{bc}	23.32±2.00^{b}
0.75%	0.00±0.00^{a}	14.72±3.68^{ab}	18.40±0.00^{b}	21.68±2.00^{b}	24.96±1.64^{bc}	24.96±1.64^{b}
1.0%	0.54±0.54^{a}	18.40±0.00^{ab}	18.40±0.00^{b}	20.04±1.64^{b}	26.60±0.00^{c}	26.60±0.00^{b}
1.25%	1.80±1.20^{a}	14.72±3.68^{ab}	14.72±3.68^{b}	20.04±1.64^{b}	23.62±2.14^{bc}	25.56±1.82^{bc}
1.50%	1.74±1.14^{a}	18.40±0.00^{ab}	18.40±0.00^{b}	20.24±1.60^{b}	20.24±1.60^{b}	22.18±2.13^{b}
1.75%	4.00±1.64^{a}	11.24±4.59^{ab}	11.24±4.59^{ab}	23.62±2.14^{b}	26.90±0.30^{c}	26.90 ±0.30^{c}
2.0%	2.80±1.71^{a}	7.56±4.63^{ab}	15.12±3.78^{b}	27.20±2.84^{b}	26.40±1.55^{b}	26.40±1.55^{c}
2.25%	4.20±1.74^{a}	11.04±4.50^{ab}	16.36±4.38^{b}	23.32±2.00^{b}	32.18±1.02^{d}	32.96±0.83^{b}
2.50%	4.60±1.88^{c}	16.36±4.38^{ab}	16.36±4.38^{b}	26.28±2.34^{b}	31.88±1.32^{d}	32.68±1.58^{d}
Pemethrin	90.00±0.00^{b}	90.00±0.00^{c}	90.00±0.00^{c}	90.00±0.00^{c}	90.00±0.00^{e}	90.00±0.00^{e}
Control	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
ANOVA	df= 11,59	df= 11,59	df= 11,59	df= 11,59	df= 11,59	df= 11,59
RESULT	F= 511.357	F= 43.411	F= 49.214	F= 137.154	F=229.285	F= 217.062
	P< 0.001	P<0.001	P<0.001	P<0.001	P<0.001	P< 0.001

*Values are means± SE

*Means followed by the same letter of alphabet within the column are not significantly different using SNK at 5% probability level

IV. Discussion

Food production and storage have been subjected to great threat from contamination, damage and destruction from storage pests. This has been a trend for many decades especially when stored food products are unprotected (Olaifa and Adenuga, 1988; Ogunkoya and Dawodu, 2014; Babarinde *et al*., 2016). Due to food shortage with respect to continued increase in the number of human population assessing the available food, there is the need to double food production and storage. Synthetic insecticides became inevitable as it is effective in the control of stored product pests such as *S. zeamais*, *C. maculatus* and *T. castaneum*. Undoubtedly, between2008-2012 over 9 billion US dollars was invested in the production and distribution of insecticides for this purpose in order to control crop pests so as to reduce food shortage (Atwood and Paisley-Jones, 2017). Unfortunately, the use of these chemicals leave in their wake greater damage to human lives, animals and the collapse of the ecosystem (Fantke *et al*., 2012; Ojo, 2016). The consumption of foods protected with synthetic insecticides have exhibited traces of chemical remnants which have been implicated in the cause of some reproductive health and other immune system breakdown leading to death in some cases (Abdullahzadeh *et al*., 2015; Mengistie *et al*., 2017). A great many challenges to finding alternative in plant materials which will control stored products pests, non- poisonous to man, animals and his environment has been posed to the academia. Powders of *R. officinalis* was obtained from the leaves of the plant. The leaves was subjected to air drying in the laboratory and further drying was carried out in an oven for 48 hours at a temperature of 40^0c. Dried leaves was ground into fine powder and subsequently kept in an air tight plastic container until ready for use for the bioassay. This is in line with the processing of Rosemary leaves into powder in order to determine its effect on glucose level, lipid profile and lipid perodoxation (Borhan *et al*., 2013; Labban *et al*., 2014).

R. officinalis was effective in the control of adult *S. zeamais* due to the rate of toxicity to the beetle and subsequently the protection of the seeds in storage from its infestation and destruction. Powders of *R. officinalis* applied at 0.25% (wt/wt) dose or more to protect maize seeds was significantly toxic to adult *S. zeamais* when compared with the control. Although. 0.25%wt /wt produced the lowest toxicity. This is in line with powders from plants with insecticidal properties such as *R. officinalis*, *O. gratissimum, P. guineense,* and *D. tripetala,* used for the protection of stored cowpeas and rice (Olotuah, 2013; Philip-Attah, 2014; Ojiako *et al*., 2018; Dawodu *et al*., 2022). In this experiment, the report of toxicity on *S. zeamais* has provided more information on the insecticidal qualities present in *R. officinalis* which has added to the wealth of knowledge needed to develop plant base insecticides.

R. officinalis leaf powder produced significant toxic effect against adult *C. maculatus* and subsequently cowpea seeds was protected from destruction. At all periods of observations, Permethrin produced significantly higher toxicity than the other doses of *R. officinalis* and the control treatment. This shows that the synthetic insecticide was more toxic to the beetles than the botanical insecticide. This is in line with previous research that synthetic insecticides are more toxic to stored products insects than botanicals and do have the tendency to outlive their purpose which may be detrimental to consumers (Idoko and Adesina, 2013; Babarinde *et al.,* 2016; Dawodu *et al*., 2021b).

The potential toxicity of *R. officinalis* leaf powder against adult *T. castaneum* in stored Sorghum recorded a significantly toxic effect at ($P \leq 0.001$). It protected seeds of Sorghum in storage from damage which may be caused by the beetle. This is in line with the report of *R. offinalis* oil as it was lethal to larvae of *Psuedaletia unipuncta* and *Trichoplsia ni*. This is due to the presence of active ingredients present in the oil which are similar to those of commercial insecticides used for protection of stored product insects (Isman and Machial 2006; Dawodu *et al*., 2021b). Pemethrin produced the highest observed toxicity across the period of observation which was significantly higher than the toxicity produced by all the doses of *R. officinalis*. Although, there was no significant differences among the doses of *R. officinalis* but 0.25% wt./wt. produced the lowest toxicity at these periods which was not significantly different from the toxicity of the control treatment between 24 and 72 HAT. In all, dose 2.50% wt /wt recorded a progressive highest increase in toxicity with increase in hours after treatment between 24 and 72 hours when compared with other doses in the experiment.

V. Summary

R. officinalis has proved to be toxic to stored product insects of maize, cowpea and sorghum. These beetles have constituted to be a nuisance in the food programme of man as they give stiff competition with man through damage and destruction of stored food. The results from this research have shown a variation of toxicity levels to the three pests under investigation and have shown that *R. officinalis* can effectively control damages and destructions from *S. zeamais*, *C. maculatus* and *T. castaneum* in the storage. It is hereby recommended that powders from *R. officinalis* are good insecticides for the control of stored products pests.

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