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Effects of *Tephrosia vogelii* and rabbit urine formulation on insect pests and yields of cowpea Singida, Tanzania

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Abstract

An experiment was conducted at Jineri village, Singida rural to evaluate the effects of 10%(w/v) *Tephrosia vogelii* (T) and 50%(v/v) rabbit urine (U) on insect pests and yield of cowpea in Singida Tanzania from February 2021 to June 2021. The T and U treatments were mixed in 10% (v/v) sunflower oil, and sterile water and synthetic pesticide (karate 2.5EC) were used as negative and positive control respectively. The experiment was laid down in a complete randomized block design (CRBD) with three replications. The results indicated that there was significant difference ($P \leq 0.001$) on insect pest counts between plots sprayed with different treatments. The plots treated with positive control exhibited smaller mean number (4, 7 and 5) followed by OUT formulation (11, 8 and 4) for aphid, leaf miner, and pod borer respectively, at flowering stage, i.e., seventh week after germination. Plots sprayed with sterile distilled water had higher mean numbers of insect pests (25, 20 and 12) for aphid, leaf miner and pod borer respectively, compared with other treatments at flowering stage. The resilts indicate that the OUT formulation improved yield to a degree comparable with that of positive control, as evidenced from their close grain yield values of 794 kg/ha and 846 kg/ha respectively, which are both significantly higher than 483 kg/ha of negative control. Based on the results, the OUT-formulation is recommended for managing cowpea pests in the field.

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Introduction

Cowpea *Vigna unguiculata* (L.), (Fabaceae) is a herbaceous legume crop that is native to Africa but currently grown all over the world in areas with tropical climate (Herniter *et al.*, 2020). It is capable of thriving even in regions with limited rainfall due to its deep root systems which maximize plant water uptake and minimize epidermal conductance limiting water loss(Mitra, 2001). Moreover, cowpea is mostly preferred by farmers since it is a nutritionally important food crop especially in drier areas (Singh *et al.*, 2003). Its fresh leaves are used as vegetable for human consumption and as animal fodder (Owade *et al.*, 2020). Mineralogically, cowpea has been cited to contain zinc and iron and phenolic compounds that are considered important for human health (Carvalho *et al.*, 2012). Dry grain of cowpea contains 23–32% protein and essential amino acids(Carvalho *et al.*, 2017). It also has additional multi-functional characteristics, such as maintaining the soil-ecology balance through nitrogen fixation by facilitating a symbiotic relationship with nodulating bacteria (Qin *et al.*, 2016).

The production of cowpea requires rainfall ranging from 400 to 700mm per annum and temperature between 30-35°C (Kebede & Bekeko, 2020). In Tanzania cowpea is grown in coastal regions, also Mwanza, Dodoma, Tabora, Singida, and Shinyanga (Hella *et al.*, 2013). Cowpea growth is negatively affected by various factors such as poor soil fertility, drought, diseases, and pests (Gungula & Garjila, 2005), among these, pests are considered to be a major factor for the reduction of cowpea yield (Oyewale & Bamaiyi, 2013). To carb the challenge, cowpea farmers have relied on extensive use of synthetic pesticides which, though effective, are known to cause environmental pollution as well as human health issues, threatening ecosystems (Oyewale & Bamaiyi, 2013). Some synthetic insecticides such as pyrethroids and DDT affect the nervous system, acetylcholine receptors and voltage gated-sodium pump, in human beings (Rani *et al.*, 2021). The growing awareness of the detrimental effects of synthetic pesticides on the environment and human health has recently attracted the attention of farmers and scientists on agro ecological farming which includes the use of pesticidal plant extracts in controlling insect pests. Botanical pesticides have been advocated as environmentally friendly approach of fighting pests (Mkindi, 2016) as they cause less harm to the environment. Some of the botanical pesticides reported include *Azadirachta indica* in insect control (Damalas & Koutroubas, 2020), *Tephrosia vogelii* and *Petiveria alliacea* which at 20% and 10% v/v respectively extract significantly protected cowpea pods and grains from the damage (Alao *et al.*, 2011). Several authors have also reported the use of plant oils against insect pests.

On various insect species, plant bio-pesticides based on essential oils (Eos) possess poisonous, repellent, and insecticidal properties (Ibrahim, 2019). Sunflower oil at 10ml significantly reduces the lifetime of C. rhodesiunus on stored cowpea (Rajapakse & Van Emden, 1997). In addition, fermented rabbit urine spray has been found to help in the management of insects and other pests, including aphids, moths, leaf miners, caterpillars, and mites (Corbeels *et al.*, 2019).

However, despite their environmental friendliness, botanical extracts are less effective compared to synthetic pesticides when used as a single extract product. Some studies have shown that combining two or more plant extracts may result in synergy (Oparaeke *et al.*, 2005). Since rabbit urine, *T. vogelii* extract, and sunflower oil have been reported to have insecticidal properties, their combination may result in synergistic improvement in pesticidal performance. This study aims to investigate the synergistic effects of a combination of *T. vogelii*, rabbit urine, and sunflower oil on cowpea growth vigour, yield, and yield component.

Materials and methods

Study site

This study was conducted in Singida Region, Northern Tanzania, between latitudes 30 52' and 70 34' south, longitude 330 27' and 350 26' east (Salaam, 2017).

The region is characterized by semi-arid climate, with an annual rainfall of about 500mm to 800mm. The temperatures in the region vary according to altitude but generally range from about 15°C in July to 30°C.

Materials collection and insecticide formulation

Tephrosia vogelii leaves were collected randomly from local uncultivated farmlands. The leaves were dried under shade for 7 days to minimize the loss of volatile compounds (Sejali & Anuar, 2011). Dry leaves were then pulverized using an electric grinder, packed in 1kg plastic bags, and stored in dark dry condition. Rabbit urine and cold-pressed cooking oil were purchased from local rabbit keepers and local factories in Singida, respectively.

The *T.vogelii* powder was soaked in an aqueous solution containing 0.1% soap to obtain an extract. Soap is used as a medium for the extraction of compounds in plant extracts (Stevenson *et al.*, 2012). The mixture was left to stand for 24 hours after which the extract was filtered through a clean cloth.

In this study, various formulations were conceptualized and tested. The tested formulations were made using the following ingredients: *T.vogelii* extract (T), rabbit urine (U), sunflower oil (O) in concentrations of 10%, 50%, and 10%, respectively. 10% was equivalent to 100ml diluted with 1000ml of water to achieve the same spraying volume. The ingredients were used individually and in combination resulting in the following formulation O, U, T, and OUT. To the formulations, dish detergent was added to break the layer between liquid and oil.

Experimental design

The experiment was set up as a complete randomized block design (CRBD) of six treatments with three replicates. Experimental plots measuring 3 x 3 m^2, with 1 m alleys left between them to prevent pesticide drift and inter-plots, were prepared using a hand hoe. Seed variety of (*Dolichos melanophthalmus*) black-eyed cowpea from Tropical Pesticide Research Institute (TPRI) were planted at a spacing of 50 and 20cm between and within rows respectively, with two plants per hole at 3-4cm deep which was later thinned to one when plants were one week old from germination. The formulations were applied to the cowpea plots in a complete randomized block design.

The performance of the formulation was assessed by quantifying insect abundance reduction and increase in yield. Yield parameters including the number of pods, 100 seed weight, and weight of the yield per plot, expressed inkg/ha, were used to assess the effect of the formulations on yield. Large insects, leaf miners, and pod borers were counted individually.

On the other hand, due to always being in very large numbers, aphid abundance was assessed by categorical index, where only large colonies with more than fifty individual aphids were counted (Tembo *et al.*, 2018). Insect identification was done in TPRI under entomologist. The performance of each treatment was compared with the control positive (synthetic pesticide) karate Lambda-cyhalothrin and control negative (with water only).

Data analysis

Differences in insect abundance and cowpea yield among treatments were assessed by analysis of variance (ANOVA) and Tukey's post-hoc Honestly Significant Difference (HSD) test was used to separate the means at the 95% confidence interval.

Results

Insect abundance

The results indicate that there is a significant difference on reduction of insect abundance ($P \leq 0.001$). The abundance of pests was low in positive control plots giving the mean numbers of (4.3, 4.7 and 5.0), followed by the OUT formulation (11, 8 and 4.3), compared to the mean abundance of control negative (24.7, 19.7 and 12.0), for aphid, leaf miner and pod borer respectively, during flowering. Generally, the OUT formulation showed a higher abundance of insect pests than the synthetic pesticides but lower than the negative control and individual ingredients (O, U and T). Table1a & b for aphids and tables 2 & 3 for leaf miner and pod borer respectively.

Table 1a. Aphid abundance reduction trend recorded weekly per treatment on cowpea after germination.

Treatment	WK2	WK3	WK4	WK5	WK6	WK7	WK8
C-	4.0±1.2a	8.6±0.7b	11.0±0.6a	15.0±0.6a	18.3±0.8a	24.7±0.6a	23.0±0.6b
C+	3.3±0.8a	5.3±0.7a	5.6±0.7c	5.7±0.8d	5.3±0.3d	4.3±0.3d	6.0±0.6e
O	4.0±1.2a	8.0±0.6ab	9.0±0.6ab	12.3±0.8abc	17.0±0.6ab	24.0±1.2a	30.3±0.9a
U	6.7±1.2a	8.0±0.6ab	11.0±0.6a	13.7±0.3ab	14.3±0.7bc	12.7±0.3bc	17.7±1.2c
T	4.0±0.6a	5.3±0.7a	7.6±0.3bc	11.0±0.5bc	12.7±0.8c	14.0±0.6b	16.0±0.6c
OUT	4.6±0.8a	7.0±0.6ab	7.0±0.8bc	10.0±0.6c	12.0±0.6c	11.0±0.6c	11.3±0.7d
Mean	4.4	7.1	8.6	11.3	13.3	15.1	17.4
CV	37.1	15.5	11.9	11	7.5	6.4	8.1
LSD	1.9	1.9	1.9	2.2	1.7	1.7	3.4
P. value	0.273	0.013	<.001	<.001	<.001	<.001	<.001

The values presented are means ± SE and significant different at P.value, Means followed by the same letter in a column are not significantly different

Table 1b.

Treatment	WK9	WK10	WK11	WK12	WK13	WK14	WK15
C-	23.3±2b	24.7±0.7b	34.7±0.7a	37.0±0.6a	34.0±0.6a	37.0±0.6a	36.3±0.9a
C+	4.0±0.6e	5.7±0.9e	5.3±0.3f	6.3±0.3e	4.0±0.6e	4.0±0.6f	3.0±0.6d
O	32.7±0.3a	31.0±0.6a	29.0±0.6b	27.3±1.4b	28.7±1.2b	32.0±1.2b	32.0±1.5a
U	20.3±0.9bc	22.0±0.6b	21.3±0.9c	24.0±0.6b	21.3±0.9c	20.3±0.9c	20.7±1.2b
T	15.3±0.3c	17.0±0.6c	16.7±0.9d	19.0±0.6c	18.3±0.9c	16.0±0.6d	16.3±0.9b
OUT	9.3±0.9d	12.0±0.6d	11.0±0.6e	14.0±0.6d	10.0±0.6d	10.0±0.6e	10.0±0.6c
Mean	17.5	18.9	19.7	21.3	19.4	19.9	19.7
CV	10.7	6.1	4.0	6.0	6.1	6.1	8.8
LSD	3.4	2.1	2.3	2.1	2.1	2.1	3.2
P. value	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Table 2. Analysis of variance on the Abundance of leaf miner recorded weekly per treatment on cowpea after germination.

Treatment	WK2	WK3	WK4	WK5	WK6	WK7	WK8	WK9	WK10
C-	4.3±1.2a	4.3±0.9a	7.0±0.6ab	15.0±1.2c	16.0±1.2d	19.7±1.5e	14.0±1.2b	17.0±1.2c	20.0±1.2c
C+	3.0±1a	3.0±0.6ab	5.0±0.6a	4.0±0.6a	3.3±0.3a	4.7±0.3a	4.3±0.9a	3.7±0.7a	5.3±0.9a
O	9.0±0.6b	4.3±0.9ab	10.0±1.2b	16.7±0.9c	15.0±0.6d	11.7±0.9c	15.7±1.5bc	13.3±0.9bc	11.3±1.5b
U	3.7±0.3a	4.7±0.3ab	8.0±1.2ab	10.0±0.6b	13.3±0.3cd	16.3±0.9d	19.0±0.6c	16.7±0.9c	14.0±0.6b
T	5.0±0.6a	5.7±0.3b	5.7±0.9a	8.0±0.6b	10.7±0.3bc	10.0±0.6bc	7.3±0.9a	11.0±0.6b	9.3±0.9ab
OUT	2.7±0.3a	5.0±0.6ab	8.7±0.9ab	11.0±0.6b	8.0±0.6b	8.0±0.6b	8.0±0.6a	5.7±0.9a	6.0±0.6a
Mean	4.6	4.5	7.4	10.8	11.1	11.7	11.4	11.2	11.0
CV	25.9	16.2	18.8	12.9	10.1	9.0	15.2	13.2	13.2
LSD	2.2	1.3	2.5	2.5	2	1.9	3.1	2.7	2.6
P. value	<.001	0.022	0.011	<.001	<.001	<.001	<.001	<.001	<.001

Table 3. Pod borer abundance.

Treatment	WK5	WK6	WK7	WK8	WK9	WK10
C-	10.0±0.6c	15.0±0.6d	12.0c±0.6c	12.0±0.6c	14.0±0.6e	14.0±0.6d
C+	3.7±0.3a	4.3±0.3a	5.0a±0.6a	2.7±0.8a	3.0±0.6a	2.3±0.3a
O	6.0±0.6b	7.3±0.9c	10.0±0.6bc	11.0±0.6c	10.3±0.9d	10.0±0.6c
U	7.3±0.3b	5.3±0.3abc	5.7a±0.9a	6.0±0.6b	6.0±0.6bc	7.6±0.3b
T	5.6±0.3ab	7.0±0.6bc	6.7±0.9a	5.0±0.6ab	7.7±0.3cd	6.3±0.3b
OUT	5.0±0.6ab	5.0ab±0.6ab	4.3a±0.3a	4.0±0.6ab	3.3±0.3ab	4.0±0.3a
Mean	6.3	7.3	7.3	6.6	7.4	7.3
CV	14.1	11.1	16.6	14.0	13.9	10.3
LSD	1.6	1.5	2.2	1.7	1.9	1.34
P. value	<.001	<.001	<.001	<.001	<.001	<.001

The values presented are means ± SE and significant different at P.value, Means followed by the same letter in a column are not significantly different.

Crop yield and yield components variations.

Yield parameters (number of pods, 100 seed weight, and yield inkg/ha) variations are presented in Fig. 1,2 and 3. The number of pods per plant varies among the applied formulations with negative control showing the minimum performance.

The plots sprayed with OUT formulation, combining 50%, 10%, and 10% of rabbit urine, *Tephrosia vogelii*, and sunflower oil (OUT), respectively, did not show significant difference with synthetic pesticides in the number of pods (Fig.1).

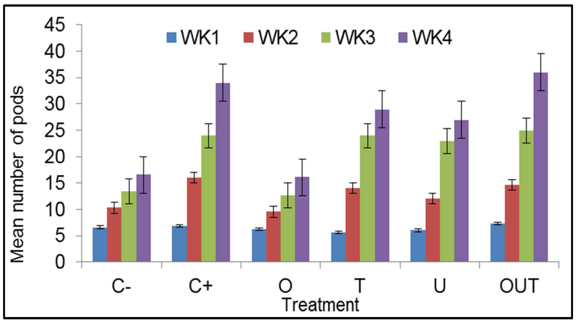

Fig. 1. Number of pods per week.

The 100 seed weight in grams for the formulations under investigation was measured and ranges between 22g and 42g (Fig. 2) with the OUT formulation giving results comparable with the synthetic pesticide, OUT = 40.3g/100 and C^{+}= 42 g/100. The differences in average of 100 seed weight were significant across treatments (C^{+}, C^{-}, T, U, O and OUT) as established by one-way ANOVA ($F_{8,27}$ = 1269, p < 0.001)

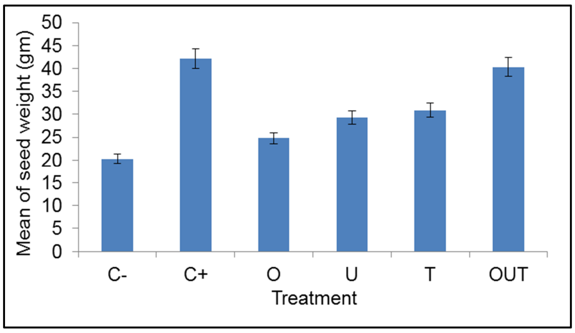

Fig. 2. 100 seed weight.

In terms of seed yield (Kg/ha), OUT gave comparable results with control positive (synthetic pesticides) Fig. 3. The OUT sprayed plots produce grain yield of (846.1kg/ha), closely followed by C^{+} (794.6kg/ha), and T (662.2kg/ha), with C^{-} plots giving the minimum yield (483.1kg/ha). Although un-combined treatments O, U, and T showed better results than the control negative, they were far below the synthetic pesticide and OUT formulation in performance.

The OUT combination was significantly effective than formulations made from its ingredients and other combination, evidencing synergy among O, U, and T. It is observed that, despite insect pest abundance on crops treated with the OUT formulation being significantly higher than observed with the synthetic control, crop yield parameters indicate comparable results.

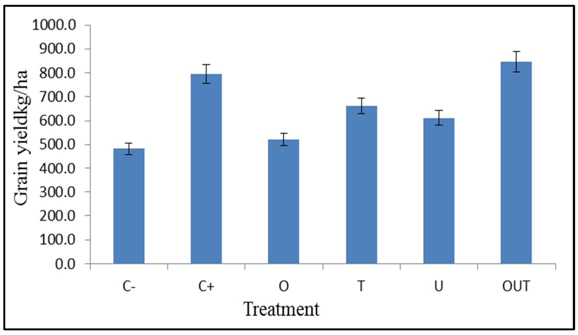

Fig. 3. Cowpeas seed yieldkg/ha.

This result shows that combining plant pesticidal extracts and rabbit urine can improve pesticidal performance due to synergistic effect.

Discussion

Insect abundance

Results in this study indicated that combining *T. vogelii* extract, rabbit urine, and sunflower oil to make an immersion (with a detergent) with anti-insect properties leads to synergistically enhanced performance compared to the uncombine formulations. The synergistic effect is evidenced by the number of times the performance is improved when the combined formulation is applied.

It was revealed that, compared to the control negative, the OUT reduced aphid, pod borers, and leaf miner abundance up to 2-3-fold. Despite the improvement, the control positive still exhibited slightly better performance (lower aphid abundance) than OUT and significantly higher than the uncombine formulations. Previous studies have reported high differences between the positive control and the botanical extract formulations on reduction of insect pests on cowpea (Tembo *et al.*, 2018). The improved performance of the OUT formulation in this study could be due to the multiple modes of action of the various bioactive compounds that it contains.

While Oil works by blocking spiracles hence suffocating the insect, rabbit urine contains ammonium compounds that give it a characteristic pungent smell (MOHAMMED, 2016) which exerts repellence on insects. Corbeels *et al.*, 2019, reported that fermented rabbit urine spray helps to manage insects and other pests, including aphids, moths, leaf miners, caterpillars, and mites. On the other hand, phytochemical analysis of *T. vogelii* has indicated the presence of the rotenoids deguelin, tephrosin, and rotenone (with deguelin being the most abundant) (Stevenson *et al.*, 2012).

The retinoids are the only group of flavonoids known to be strongly toxic to insects (Mkindi *et al.*, 2019). The mode of action of rotenoids is to depress cellular respiration of insects by interfering with electron transport in the oxidation of NADPH to NADP+ by cytochrome b, which then inhibits the mitochondrial oxidation of Krebs cycle intermediates. There is a dramatic fall in oxygen uptake on applying rotenone to insect cells(Zhang *et al.*, 2020).

Due to distinct chemotypes in *T.vogelii*, individual plants may differ significantly in rotenone, deguelin, rotenone, and tephrosin contents (Belmain *et al.*, 2012). *T.vogelii* plant with chemotype 1, has insecticidal property while flavonoids in chemotype 2 were inactive against insect(Mkindi *et al.*, 2019).Other studies (Karungi *et al.*, 2000; Mkindi *et al.*, 2017) reported the significant difference of (P≤0.01) when *T.vogelii* is used independently at 10% concentration which is greater than (P≤0.001) in this study evidencing efficacy in combination with sunflower oil and rabbit urine in terms of reducing pest damage and increasing yield. The lower abundance observed with the positive control may be due to the high efficiency of the mode of action of lambda-cyhalothrin and its high affinity with its receptor. Lambda-cyhalothrin acts by penetrating the insect cuticle to disrupt nerve conduction by keeping the sodium channel activation gate in an open position causing prolonged excitation of nerve fibres resulting in paralysis and death (He *et al.*, 2008).

This study is in line with Olaitan *et al.*, 2011 reported that the effectiveness of two plant extracts *Tephrosia vogelii* and *Petiveria alliacea* in mixture as insecticide significantly suppressed insect pest's population compared with control. While other studies (Tembo *et al.*, 2018) showed effectiveness of *Lippia javanica*, *T. vogelii*, and *T. diversifolia* in reducing insect pests count on cowpea similarly to synthetic pesticide, results in this study revealed the efficacy of combining *T.vogelii,* sunflower oil and rabbit urine on insect pests management comparable to synthetic pesticide.

Yield and yield components.

Despite higher insect pest abundance of the OUT formulation compared to the synthetic treatment, the cowpeas grain yields were comparable. The yield parameters namely number of pods, 100 seed weight, and grain yield in kilogram per hectare of OUT treated plots are comparable with those of the positive control. The fact that rabbit urine contains potential nutrients including 1.05% nitrogen, 0.01% phosphorus, 0.85% potassium, and 0.12% calcium, a better nutrient composition than even commercial foliar fed fertilizers, its application may have contributed to the improved crop yield (Mutai, 2020). In addition, the comparable yield of OUT to the control positive despite higher insect abundance may have been contributed by its ability to maintain the diversity of beneficial insects useful for pollinations (Wezel *et al.*, 2014). The results in this study are in line with the finding of (Alao *et al.*, 2011) who reported that the combination of *Tephrosia vogelii* and *Petiveria alliacea* at 10% reduced pod damage and increased grain quality compared with positive control and singly applied formulations. The significantly high number of pods in OUT formulation is due to the contribution of plant extracts to plant nutrition as a foliar fertilizer, hence its use in crop protection have maintained crop yield apart from insect pest control similarly supported by (Mkindi *et al.*, 2017) and (Kayange *et al.*, 2019) reported that sprayed plots with *T. vogelii* and *T. candida* had the highest pod number at higher extraction concentration.

Conclusion

This study investigated the existence of insecticidal synergy in combining *T. vogelii* extract, rabbit urine, and sunflower oil. The results revealed that combining *T. vogelii* with rabbit and sunflower oil improves pesticidal performance due to the synergistic effect. Despite slightly higher insect abundance observed on crops treated with OUT combination than synthetic insecticides, crop yields showed comparable results, providing a valuable alternative to synthetic insecticides. The study demonstrated that blending pesticidal botanical extracts with animal urine could form the basis for a successful formulation and commercialization of biopesticides for environmentally friendly insect-pest management in the whole concept of agro-ecological farming.

Recommendations

Further studies are required for validation of the efficacy of the formulation in other crops, locations, and spraying schedules for optimum grain yield.

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