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Invitro Evaluation of Native Herbal Pesticide against Potato Pest (White Grub) Collected From Skardu District

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Abstract

Farmers in Gilgit-Baltistan are extensively using pesticides to control white grubs, leading to ecological concerns. Field surveys revealed that amount potato tuber damage varied by locality and variety, with the highest infestation (48%) in a non-registered potato variety at Katpana and the lowest (25%) in the Roko variety grown at Sadpara, Skardu. White grubs, also known as chaffer beetles or May-June beetles, are polyphagous pests that damage a wide range of crops. The present study evaluated the pesticidal effects of ethanol-based extracts from *Artemisia sieversiana* and *Ferula jieschekiana* under controlled conditions. Results revealed statistically significant ($p \leq 0.05$) among different concentrations of selected species. Bioassays were conducted using three concentrations (1%, 3%, and 5%) of dried plant parts. The highest mortality (90%) was recorded with 5% *A. sieversiana* after 72 hours, while *F. jieschekiana* at 5% showed 80% mortality. Repellency tests indicated maximum repellency (50%) in *F. jieschekiana* after 90 minutes. Pellets made from *F. jieschekiana* retained shape better and were more effective in repellency after 90 minutes compared to *A. sieversiana*. Overall, both species showed promising mortality and repellency effects, highlighting their potential as eco-friendly bio-pesticide alternatives to harmful chemical insecticides for sustainable pest management in potato farming.

Keywords: Artemisia; ferula; bio-pesticidal activity; white grub; mortality; repellency.

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Introduction

The northern part of Pakistan, Gilgit-Baltistan, has distinctive biodiversity due to the presence of three major mountain ranges (Abbas et al., 2013). Potato (*Solanum tuberosum* L.) is one of the most important vegetable crops for human nutrition. It is cultivated in over 100 countries and ranks as the fourth largest food crop worldwide. In Pakistan, potato is extensively grown, with an average production of 7.9 million tonnes per hectare globally (FAD STAT, 2022).

White grubs are polyphagous and among the most destructive and troublesome insect pests, significantly reducing potato production (Chandel et al., 2015). They cause severe damage to a wide range of plants, including fruit and forest trees, nurseries, vegetables, lawns, and field crops. Both the adult and larval stages are polyphagous, feeding on numerous cultivated and uncultivated plant species (Chandel et al., 2015). In Gilgit-Baltistan, white grubs are the most damaging pest of potato tubers and a major cause of economic loss. Most species have a six-month larval stage and an average life cycle of one year (Chandel et al., 2015).

Biopesticides are environmentally safe pest control agents derived from naturally occurring plants, animals, and microorganisms as well as their by-products (such as semiochemicals, phytochemicals, and microbial products) (Mazid et al., 2011). In contrast, synthetic pesticides can make soil brittle, reduce soil respiration (Zakir et al., 2019) and decrease the activity of beneficial soil macro-organisms such as earthworms (Samada et al., 2020). Biopesticides can also be made from common natural substances like bacteria, plants, animals, and minerals. For example, baking soda and canola oil are considered biopesticides due to their pesticidal properties (RAHMAN et al., 2016; Abbott., 1925). By the end of 2001, there were approximately 195 registered biopesticide active ingredients and 780 commercial products (Marrone, 2014, Nosheen et al., 2018).

In Gilgit-Baltistan, farmers often use toxic chemical pesticides indiscriminately to control white grub populations. Although the region's diverse flora has many medicinal uses (Hayat et al., 2009) little effort has been made to isolate, identify, and develop biopesticides from local plants. Since plant materials are traditionally used for pest control in much of Gilgit-Baltistan, the region's rich biodiversity offers strong potential for establishing botanical pesticide formulation units (Ayub et al., 2019; Hussain et al., 2011). For instance, *Sophora alopecuroides*, found abundantly in Skardu (Baltistan), has shown strong potential for commercialization as a natural pesticide against various insect pests (Ayub et al., 2019).

The main objectives of this study are to evaluate the potential of locally available plants as biopesticides and to screen these bio-agents by conducting repellency and mortality bioassays against white grubs under controlled conditions.

Materials and Methods

Study area

The Baltistan is located in the far north of Pakistan. Comprising ten districts including Skardu, its population, estimated at 1.5 million, is dispersed across an area of 72496 square kilometers, with a density of 10 people per km. The climatic condition of Skardu are highly variable. Winter temperatures can plunge to -21°C, while summer temperatures may soar to 35°C and fertility evaluation of mountainous soils shows optimum nutrients with no pollutants hence providing a challenging yet distinct environment for agricultural research. Just 69,480 hectares (0.96%) of the total area are used for cultivation; the remaining area is made up of rangeland, mountains, forests, lakes and rivers, and about 60,000 hectares of arable land are barren (IUCN, 2003).

Plant material and sampling

Two plan species, *Artemisia sieversiana* and *Ferua jaeschkeana*, were collected from different locations in Skardu, Baltistan, as shown in Table 1. The collected herbal plants were identified and confirmed by experts at the Department of Botany, University of Baltistan Skardu, PARC Skardu, and the NARC Islamabad Herbarium. These plants were selected for their potential to control pests. The collected material was processed into powder and formulated into pellets for testing as bio-pesticides. Mortality and repellency tests were conducted at the PARC Skardu Station laboratory against the potato pest white grub (*Holotrichia* spp.). **(Annexure A)**

Drying of medicinal plants

All plant materials were thoroughly washed under running tap water to remove dust and debris. They were then dried in the dark to preserve their active compounds. Once dried, the plant material was ground into fine powder and stored in airtight jars until further use.

Crude extracts preparation

Crude extracts were prepared in the Botany Laboratory, University of Baltistan Skardu (Figure 1) to evaluate the insecticidal properties of ethanolic extracts against white grubs. The maceration method was used for extraction. For each sample, 50 g of plant powder was placed in a conical flask containing 200 ml of ethanol. The flasks were covered with aluminum foil and left for three days, during which they were shaken two to three times daily. The mixtures were then filtered through Whatman No. 1 filter paper, and the filtrates were poured into Petri plates and left to evaporate at room temperature for 24 hours. The dried crude extracts were transferred to beakers or vials and stored in a refrigerator until required for bioassays (Ayub et al., 2019). **(Annexure B)**

Collection and preservation of pests

White grubs were collected from various sites, including PARC Skardu Station, Hussain Abad Skardu, and the Agricultural Research Station Ranga, using hand-picking and pit-digging methods (Table 2). The collected insects were transferred to the PARC laboratory, placed in earthen pots, and provided with food to maintain their survival until bioassays were conducted altitude also effect the infestation and growth dynamics (Ayub et al., 2019, Laub et al., 2018, Yaqoob et al., 2017). **(Annexure C)**

Pellets preparation and Storage

Pellets of plants powder were prepared by mixing 50g of each plant (02 species) powder to the 200g of clay and mixed well by adding water as requirement figure 3. The mixture was placed in clean earthen bowl and mixed in an appropriate ratio to make pellets by using meat mincer. All pellets were allowed to dry at room temperature and stored in polythene zipped bags until use (Ayub *et al.*, 2019). **(Annexure D)**

Pellets strength

Pellet strength was tested under two conditions: fully submerged in water and under moist conditions, following the method described by (abbot et al. 1925).

Bioassay for mortality

Toxicity tests were conducted to assess the efficacy of *Ferula jaeschkeana* and *Artemisia sieversiana* extracts at concentrations of 1%, 3%, and 5%. Potato slices were treated with each concentration and placed in earthen bowls containing 10 insects. Control slices were dipped in distilled water and placed in bowls with 10 insects. The soil in the bowls was kept moist by spraying water twice daily. Dead insects were counted after 24, 48, and 72 hours. Mortality percentage and corrected mortality were calculated using formula present by Niber (1994) and Abbot (1925) respectively.

Percent larval mortality = $\frac{\text{Number of dead larvae}}{\text{Total number of treated larvae}}$ x 100 (Eq. 1)

Corrected mortality = %T-%C/100-%C (Eq. 2)

Where, T, % mortality in treatment and C, % mortality in control. **(Annexure E)**

Bioassay for repellency

Repellency tests were conducted in boxes measuring 28 × 9 cm. One potato weighing 80–95 g with 10 g of pellets was placed on one side of the box, while a potato of the same weight without pellets was placed on the opposite side. Ten white grubs were released in the center of the box. The number of grubs moving to each side was recorded after 30, 60, and 90 minutes. Repellency percentage was calculated according to the method described by Ayub et al. (2019).

Data analysis

Data were analyzed using Microsoft Excel (2010) to compare treatments. Descriptive statistics, ANOVA, and LSD tests were performed to determine significance at the 0.05 level using the statistical software package Statistix 8.1 (USA).

Results

Potato damage

Potato damage and pest infestation data were collected from three localities: Hussain Abad, Katpana, and Sadpara (Table 3). The highest infestation, 48%, was recorded in a non-registered potato variety at Katpana. The lowest infestation, 25%, was found in the variety Roko at Sadpara. At the Katpana Agriculture Research Complex, the non-registered variety showed 42.86% infestation, while the variety Lady Rosita had no infestation. **(Annexure F)**

+ 20-30%, ++30-4-%, +++ ≥40

Pellets strength in fully dissolve in water

Pellets stored in sealed polyethylene bags kept their shape for at least two months at room temperature, showing good storage stability and ease of transport. In water, *Artemisia* pellets dissolved up to 95% after one hour and thirty minutes, with the lowest dissolution of 25% after thirty minutes. *Ferula* pellets dissolved up to 90% after one hour and thirty minutes, with the lowest dissolution of 20% after thirty minutes (Table 4). **(Annexure G)**

Pellets strength under moist condition

Under moist conditions, pellet strength decreased more quickly. The lowest value, 0%, was recorded after thirty minutes, and the highest value, 40%, was recorded after one hour and thirty minutes (Table 5). **(Annexure H)**

Mortality (%) of white grub

Toxicity tests were conducted using ethanolic extracts of *A. sieversiana* and *F. jaeschkeana* at concentrations of 1%, 3%, and 5% (Figure 3). After 24 hours, the 5% *A. sieversiana* extract caused the highest mortality at 50%. Lower mortality rates of 20% and 30% were recorded for 1% and 3% extracts of *A. sieversiana* and *F. jaeschkeana*. After 48 hours, mortality for the 5% *A. sieversiana* extract dropped to 20%, and the lowest mortality of 10% was recorded for 1% *Ferula*. After 72 hours, mortality ranged from 0% for 1% *Ferula* to 20% for 5% *A. sieversiana* and 3% and 5% *Ferula*. Across the trial, the highest overall mortality was 90% for 5% *A. sieversiana*, followed by 80% for 5% *F. jaeschkeana*. The lowest was 30% for 1% *F. jaeschkeana*.

Multiple Comparison Test

The LSD multiple comparison test showed that for *A. sieversiana*, the 5% extract gave the highest mortality at all-time points, while the 1% extract gave the lowest. For *F. jaeschkeana*, the 5% extract also gave the highest mortality at 24 and 72 hours, and the 1% extract gave the lowest. Mean mortality values for *F. jaeschkeana* ranged from 26.00 ± 3.06 for 1% to 49.67 ± 3.33 for 5% (Tables 6 and 7). **(Annexure I), (Annexure J)**

ANOVA effect against White Grub Mortality for (T1, T2 and T3)

ANOVA results showed significant differences ($p < 0.05$) in mortality between the two plant species at all three time intervals. At 24 hours (T1), there was a clear species-specific effect (Table 8). This difference remained at 48 hours (T2) and 72 hours (T3), confirming that the effect of species on mortality was consistent over time (Tables 9 and 10). **(Annexure K)**

Statistically significant ($p \leq 0.05$). **(Annexure L)**

Statistically significant ($p \leq 0.05$). **(Annexure M)**

Statistically significant ($p \leq 0.05$).

Repellence effects on white grub

Artemisia sieversiana pellets showed maximum repellency of 30% after thirty minutes, but this dropped to 0% after one hour and thirty minutes. *Ferula jaeschkeana* pellets also showed 30% repellency after thirty minutes but maintained 10% repellency after one hour and thirty minutes. This indicates that *F. jaeschkeana* retained repellency longer than *A. sieversiana* (*Table 11*). **(Annexure N)**

Discussion

Field surveys revealed substantial variation in white grub infestation across potato varieties and locations in the Skardu district. The highest infestation (48%) occurred in a non-registered variety at Katpana, while the lowest (25%) was recorded in the variety Roko at Sadpara. At the Katpana Agriculture Research Complex, infestation reached 42.86% in the non-registered variety, whereas Lady Rosita showed complete resistance (0%). The variety Roko exhibited moderate infestation levels, averaging 33.33% and 16.67% at different sites. These findings highlight the influence of varietal susceptibility and local environmental conditions on pest pressure (Skendžić et al., 2021).

White grubs are known polyphagous pests, attacking a wide range of crops during the monsoon season Zaki et al., 2006, including castor, chillies, groundnut, sorghum, maize, sugarcane, soybeans, and various legumes (Hadizadeh et al., 2019, Chandel et al., 2015). Both larval and adult scarabaeid stages cause significant economic losses worldwide, with adults being nocturnal feeders. The pest's broad host range and destructive feeding habits underscore the need for integrated management strategies that address both life stages (Kumar et al., 2019).

Several plant species have demonstrated potential as eco-friendly control agents against scarab beetles. *Thevetia peruviana* leaf extract and *Datura innoxia* seed extract have been reported to act as toxicants or growth regulators, significantly reducing adult *Holotrichia serrata* populations (Theurkar, 2014, Akutse et al., 2020, Motai et al., 2004). Such botanical products offer an alternative to synthetic insecticides, aligning with sustainable pest managemzent goals (Sahebkar et al., 2010).

Conventional insecticides, including neonicotinoids, organophosphates, and pyrethroids, remain widely used for white grub and wireworm control. However, their overuse has led to environmental contamination and the development of insecticide resistance. These drawbacks strengthen the case for developing biodegradable, plant-based alternatives.

Ayub et al., (2019) found that ethanolic extracts of *A. sieversiana* achieved the highest repellency (98% at 5% concentration after two hours) and that 5%

acetone extract caused 97% mortality after 72 hours. In their study, the 5% ethanolic extract produced the second-highest mortality rate (90.3%). In our experiments, maximum repellency for both *A. sieversiana* and *F. jaeschkeana* ethanolic extracts was 30% after 30 minutes—lower than Ayub et al.'s values, likely due to differences in exposure time, environmental conditions, or pest population. However, mortality results were consistent: 5% *A. sieversiana* extract achieved the highest mortality (90%) after 24 hours, followed by 5% *F. jaeschkeana* (80%). The lowest mortality (30%) was recorded for 1% *F. jaeschkeana*. These results confirm the insecticidal potential of both species, with *A. sieversiana* generally showing stronger efficacy.

Studies have linked adult white grub (*Holotrichia* spp.) presence to severe yield losses in sugarcane, maize, peas, potatoes, and groundnuts, with neem trees among the most heavily damaged hosts (Kumar et al., 2022). Our findings align with this broader evidence, reinforcing the importance of integrating botanical repellents into pest management programs to reduce reliance on synthetic chemicals and mitigate resistance development.

Conclusions

This study demonstrates that extracts of *Artemisia sieversiana* and *Ferula jaeschkeana* have significant insecticidal activity and can serve as biodegradable alternatives to chemical insect repellents for controlling white grubs. The highest overall mortality was recorded for 5% *A. sieversiana* extract (90%), making it the most effective treatment. The lowest mortality was observed for 1% *F. jaeschkeana* extract (30%). The 5% *F. jaeschkeana* extract produced the second-highest mortality (80%).

Repellency tests at different time intervals—T1 (30 min), T2 (60 min), and T3 (90 min)—showed that maximum repellency (30%) occurred after 30 minutes. The lowest repellency (10%) was recorded for *F. jaeschkeana* after 90 minutes. Pellets containing *F. jaeschkeana* powder retained repellency longer than those containing *A. sieversiana*, making them more effective at the 90-minute interval.

These findings highlight the potential of both plant species as eco-friendly pest control agents and support further research into their field application and long-term, effectiveness. Notably their adaptability to local environmental conditions marks a significant breakthrough, enabling their application in region specific integrated pest management strategies for the first time.

Recommendations

Further research should focus on the fractionation and identification of active compounds from the selected plant species to better understand their insecticidal properties. Development of preparation methods and ground application techniques is necessary to formulate an effective botanical insecticide for white grub control. Additional studies are recommended on fermentation processes, formulation optimization, phytotoxicity assessment, and application strategies to ensure efficacy and safety. Field trials using plant-based pellets in potato crops should be conducted to evaluate their control potential and determine dose-specific effects under real cultivation conditions. This research holds significant potential for future commercial applications, representing a groundbreaking advancement in the field.

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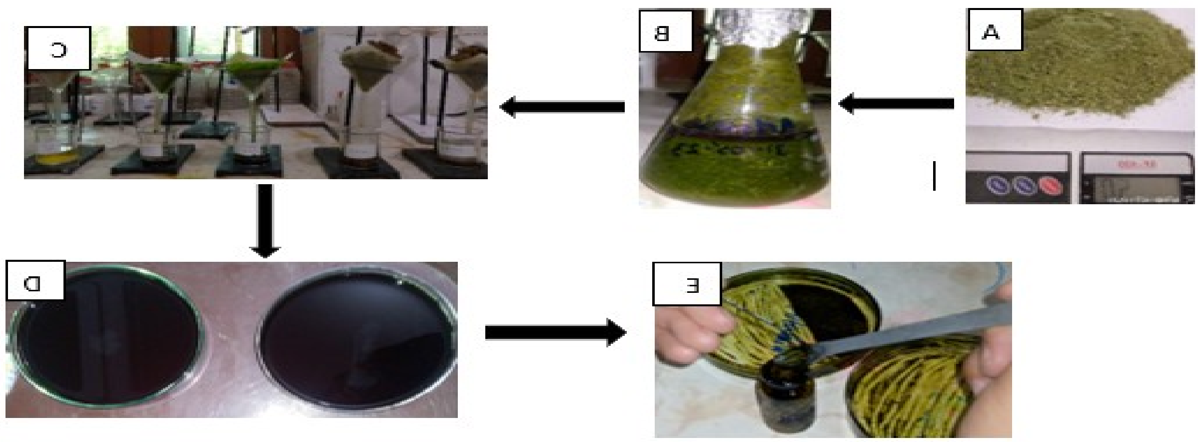
(Annexure A)

Table 1 Collection details of plant materials

S. No	Scientific Name	Local Name	Locality	District	Part used
1	*Artemisia Seversiana*	Khampa	Hussain abad	Skardu	Whole plant
2	*Ferula Jieskiana*	Sib	Sadpara/Deosai	Skardu	Root

(Annexure B)

Fig. 1 Extract preparation of *Artemisia sieversiana and Ferula jaeschkeana*



(Annexure C)

Table 2 collection details of white grubs

S. No	Location	Latitude	Longitude	Soil type
1	Hussainabad	35.296261°	75.693532°	Sandy land
2	Sadpara	35.193505°	75.624522°	Forest land
3	Katpana	35.325205°	75.577157°	Sandy land
4	Hassan Colony	35.284371°	75.628043	Farm land

(Annexure D)

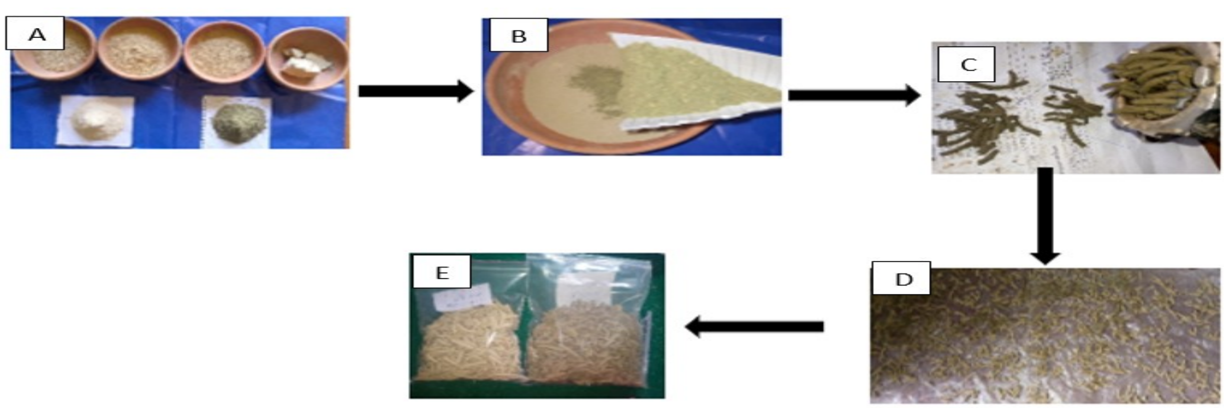


Fig. 2 Pellets preparation processes, drying and storage

(Annexure E)

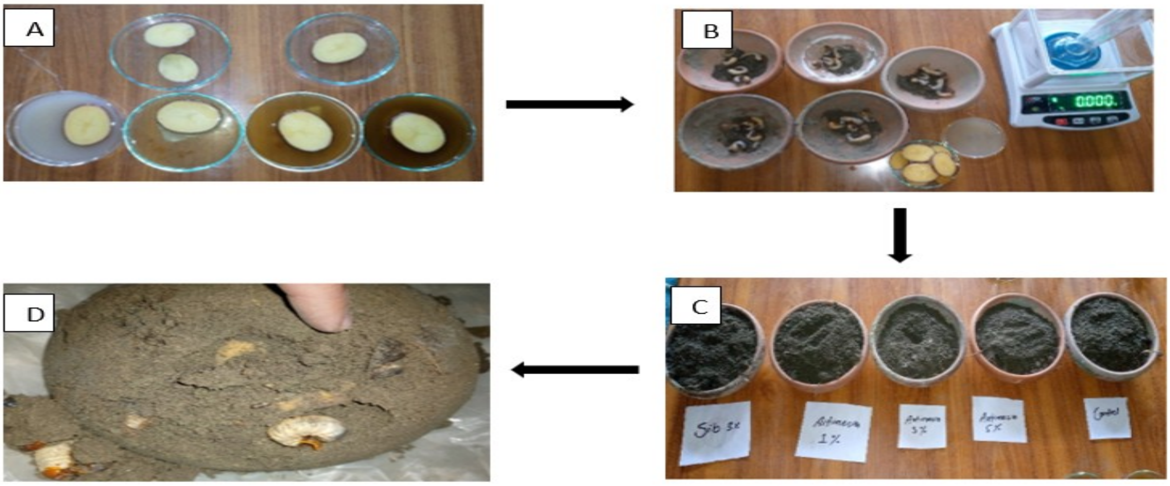


Fig. 3 White grub mortality test procedure (*in-vitro* test)

(Annexure F)

Table 3 Data about potato damage and pest infestation

S. No	Location	Respondents (Farmers)	Cultivars	Tuber infestation	Mean white grub infestation per plot +
1	**Hussain Abad**	1	Roko(R)	35%	++
		2	Non-Registered (NR)	42%	+++
		3	Non-Registered (NR)	45%	+++
2	**Katpana**	1	Roko (R)	30%	+
		2	Roko (R)	37%	++
		3	Non-Registered (NR)	48%	+++
3	**Sadpara**	1	Non-Registered (NR)	38%	++
		2	Non-Registered (NR)	30%	+
		3	Roko (R)	25%	+

(Annexure G)

Table 4 Pellets strength in fully dipped water

Pellets	30Min	60Min	90Min
Artemisia sieversiana	25%	55%	95%
Ferula jaeschkeana	20%	50%	90%

(Annexure H)

Table 5 Pellets strength under moist water

Pellets Strength in Moist Water			
Pellets	30Min	60Min	90Min
Artemisia	0%	10%	40%
Ferula	0%	20%	40%

(Annexure I)

Table 6 Descriptive statistics of *Artemisia seversiana* against white grub mortality

Species	Treatment	Mean ± SEM	MIN	MAX	SD
Artemisia seversiana	1%	19.33±4.98	10	27	8.62
	3%	36.66±3.33	30	40	5.77

	5%	46.66±3.33	40	50	5.77
Control	0%	5.00±2.89	0	10	5.00

(Annexure J)

Table 7 Descriptive statistics of *Ferula jaeschkeana* against white grub mortality

Species	**Treatment**	**Mean ± SEM**	**MIN**	**MAX**	**SD**
	1%	**26.00±3.06**	**20**	**30**	**5.29**
	3%	**37.33±3.71**	**30**	**42**	**6.43**
Ferula jaeschkeana	**5%**	**49.67±3.33**	**43**	**53**	**5.77**
Control	**0%**	**9.00±2.89**	**4**	**14**	**5.00**

(Annexure K)

Table 8 Analysis of Variance for T1 (24 hrs.) against White Grub Mortality

Source	DF	SS	MS	F	P
Rep	2	18.27	9.135		
Treat	3	765.03	255.01	18.33	0.00
Error	18	250.44	13.913		
Total	23	1033.74			

(Annexure L)

Table 9 Analysis of Variance for T2 (48 hrs.) against White Grubs Mortality

Source	DF	SS	MS	F	P
Rep	2	37	18.5		
Treat	3	880.5	293.5	19.35	0.001
Error	12	273	15.167		
Total	23	1190.5			

(Annexure M)

Table 10 Analysis of Variance for T3 (72 hrs.) against White Grubs Mortality

Source	DF	SS	MS	F	P
Rep	2	100	50		
Treat	3	520.833	173.611	16.3	0.001
Error	18	191.667	10.648		
Total	23	812.5			

(Annexure N)

Table 11 Average repellency *of Artemisia sieversiana* and *Ferula jaeschkeana* pellets

Repellency Test					
	R	**30min.**	**60min**	**90min.**	**%Repellency**
Artemiisa Pellet	R1	2	3	3	30%
	R2	3	4	4	40%
Ferrula Pellet	R1	2	4	4	40%
	R2	2	4	5	50%