RESEARCH PAPER

Studies on the Biocontrol Effect of *Mucuna Pruriens* and *Phaselous Lunatus* (Fabaceae) Seed Extracts in Protecting Stored Cowpea Against Adult *Callosobruchus Maculatus*(Coleoptera: Bruchidae)

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Abstract
Stored grain pests, especially *Callosobruchus maculatus*, makes considerable losses in agricultural products. This research assessed the insecticidal and ovicidal impacts of plant extracts obtained from *Mucuna pruriens* and *Phaseolus lunatus* on *C. maculatus*. The solvent extracts such as hexane, diethyl ether, dichloromethane, ethyl acetate, and methanol, and were then applied to cowpea seeds. The mortality of adult weevils was monitored for four days, while grain damage was evaluated through exit holes and the Weevil Perforation Index (WPI) following a duration of 49 days. The findings showed that hexane extracts from both plants were the most efficient in decreasing adult mortality and damage to grain. Mortality rates for hexane extract of *Mucuna pruriens* rose from 46.57% to 100% at elevated concentrations, and *Phaseolus lunatus* hexane extract exhibited a comparable pattern. Methanol extracts showed the lowest toxicity. Moreover, the hexane extracts notably diminished grain damage, with increased concentrations resulting in more significant damage, as shown by the WPI. These results indicate that plant extracts, especially those based on hexane, may serve as effective substitutes for chemical insecticides in managing *C. maculatus* in stored grains.

Keywords: *Callosobruchus maculates,* adult emergence, grain damage and weevil perforation index (WPI)

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Introduction

Pests that infest grain storage, especially weevils, inflict considerable harm on stored grains, poses major economic losses worldwide [1]. A significant pest that harms stored grains is the *Callosobruchus maculatus* (Fabricius), widely referred as the cowpea weevil, impacting pulses and various legumes [2]. Chemical insecticides are extensively utilized to manage these pests, but their deleterious impacts on human health, non-target species, and the environment have sparked greater interest in alternative pest management techniques [3]. Plant extracts have attracted interest as environmentally friendly options because of their insecticidal effects, usually linked to the presence of bioactive compounds like alkaloids, flavonoids, and terpenoids acting as biocontrol agents [4]. The cowpea beetle *C. maculatus* poses severe risks to stored grains, sometimes causing severe damage and economic loss [5]. To combat this invading pest, traditional methods are in practice that involves usage of locally available materials for treatment [6]. Certain medicinal plants effectively reduce oviposition, egg hatchability, and adult emergence. Research reveals that plant extracts and essential oils contain insecticidal compounds, with 1005 plant species identified for their insecticidal activities, pest management properties, repellents, antifeedants, attractants, and growth-inhibiting properties [7].*Mucuna pruriens*, a wild legume, and *Phaseolus lunatus* are recognized for their capabilities in pest control. In recent years research has indicated that extracts derived from seeds of these plants have insecticidal properties, which may be applied to safeguard stored grains [8,9]. The efficacy of plant extracts differs based on the solvent employed, as non-polar solvents such as hexane, frequently yield superior outcomes compared to polar solvents like methanol

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[10]. This research examines the effectiveness of various solvent extracts from *Mucuna pruriens* and *Phaseolus lunatus* in minimizing grain damage and managing *C. maculatus* infestations, evaluating their influence on grain quality and pest mortality.

Materials and Methods

Collection and preparation of plant extracts

The *Phaselous lunatus* seeds are procured from local market, Chennai and seeds of Mucuna *pruriens* were collected from Kolli Hills, Eastern Ghats, Tamil Nadu, India. The seed materials were taken into the lab and shade dried. The dried and sound seeds were ground with an electric blender and sieved in lab sieve for the mesh size of 1.00mm (IS Sieve IS: 460-1962) , and the fine powder was employed for extraction with the Soxhlet apparatus. The powdered seed materials were extracted sequentially with increasing polarity of solvents, including hexane, diethyl ether, dichloromethane, ethyl acetate, and methanol extract. The crude extract residues were thoroughly dried to ensure complete evaporation of solvent, and then collected in brown vials and stored at 4°C. The diluted concentration was utilized in the following studies.

Insects rearing

Callosobruchus maculatus was collected in the local grain market at Thiruttani, Tamil Nadu India. Prior to the experiments, the bruchids were reared on cowpea in the laboratory for subsequent 2 generations. The insect culture was done in a climate chamber maintained at 28 ±2ºC with 12h photo period at an ambient RH (75±5%). For the experiments, newly emerged (4 h) insects were used. While experiments, the day of death of the adult beetles was determined as the day the antennae and legs did not move upon gentle disturbance with camel brush.

Beans

Cowpea (*V.unguiculata*) of the selected variety were stored in a freezer at -18ºC for a week and subsequently dried at 60ºC for about a week to ensure the absence of viable insects without using any chemicals. The beans were stored in airtight plastic containers at room temperature before use. Only sound quality beans were taken for experimentation as assessed by its physical examination illuminated chamber.

The toxic effects of seed extract on adult *C. maculatus* were assessed using Petri dishes (9 cm in diameter) containing 25g of cowpea at concentrations of 100, 200, 300, 400, and 500 mg/kg. The solvent extracts of seeds was evenly distributed on the cowpea using a glass rod and gently agitated for 5-10 min to ensure uniform coverage. The dishes were left open for about 30 min to allow any solvent traces to evaporate. Afterwards, 20 newly emerged adult *C. maculatus* were introduced into each dish, and mortality was monitored on daily basis for consecutive four days. Grains treated with solvent alone served as the control. Adults were considered dead if there are no responses were observed after being gently probed with a camel brush.

Effect of plant extract on adult emergence and grain damage

In another set of experiment involved with leaving the infected and treated grains for 49 days (7 weeks). At the end of this period, damage due to weevil was assessed by counting the exit holes as a measure of grain damage. Grains with exit holes were counted, and the percentage damage (PD) and Weevil Perforation Index (WPI) were calculated as per the methods of Adedive and Ajayi (1996) and Fatope *et al*. (1995), respectively [11,12].

PD = (Total number of treated grains perforated / Total number of grains) x 100

WPI = (Percentage of control grains perforated + Percentage of treated grains perforated) x 100

Ovicidal activity

The ovicidal activity of seven essential oils was tested on different developmental stages of *C. maculatus*. Ten gravid female beetles were placed on cowpea seeds, and the number of eggs laid was counted. After 72 hours, the cowpea seeds with eggs were dipped into the essential oils (at 100 and 200 ppm concentrations) for 30 seconds, followed by air-drying. The hatching rate was then assessed by counting the number of hatched eggs [11-13].

Statistical analysis

Statistical analysis of the data was done using SPSS 17.0 version software package. The significant of the results were assessed at $P<0.05$ level.

Effect of plant extract on weevil mortality

Results and Discussion

Table: 1. Effect of Plant extract (*Phaselous lunutus*), Mean Mortality on adult weevils of *C.marculaltus**

Plant Name	Mean mortality(±S.E) (%) at 1-4 days post treatment				
Phaselous lunutus	Conc.ppm	Day1	Day2	Day3	Day4
Hexane	100	42.23 ± 0.98^{a}	58.03 ± 1.26^{b}	62.80 ± 1.34^{ab}	74.69±1c
	200	53.67 ± 0.46^{c}	63.93 ± 1.34^{c}	65.19 ± 1.13^{e}	76.02 ± 1.25^{c}
	300	57.73 ± 0.67^{cd}	65.71 ± 0.55^{c}	72.36 ± 0.39^{d}	79.17 ± 0.12^{d}
	400	58.07 ± 0.69^{cd}	67.18 ± 0.31^{cd}	73.24 ± 0.43^{d}	86.04 ± 0.19^{e}
	500	$59.0\ 5\pm 0.12^{cd}$	$69.0\ 1\pm 0.87^{c}$	84.57 ± 0.78^{g}	94.22 ± 0.38^{g}
Diethyl ether	100	54.61 ± 0.84^{c}	58.71 ± 0.93^{b}	60.85 ± 0.73^{a}	81.99 ± 0.72^{de}
	200	50.24 ± 1.01^{bc}	53.10 ± 0.78^{c}	63.74 ± 0.77^{ab}	83.15 ± 0.74^{de}

	300	48.98±0.93^{b}	49.76 ± 0.85^{b}	64.44±0.87^{ab}	94.28±0.74^{f}
	400	47.98 ± 1.13^{b}	51.71 ± 0.96^{bc}	66.79±0.67^{ab}	99.94±0.98^{g}
	500	48.20 ± 1^{b}	58.99 ± 0.82^{d}	71.20±0.60^{d}	100.00±0.30^{g}
Dichloromethane	100	49.14 ± 0.54^{b}	59.83±0.84^{bc}	62.62±0.81^{ab}	69.01±0.51^{b}
	200	55.42 ± 0.70^{c}	57.48±0.52^{b}	59.49±0.66^{a}	73.95±0.48^{bc}
	300	53.71 ± 0.84^{c}	57.53±0.58^{b}	65.88±0.79^{e}	84.43±0.78^{d}
	400	52.86 ± 0.81^{c}	58.88±.56^{b}	66.22±0.64^{e}	86.31±0.81^{e}
	500	54.67 ± 0.60	61.39±0.74^{bc}	68.82±0.72^{e}	97.07±0.66^{g}
Ethyl acetate	100	47.76 ± 0.53^{b}	45.69±0.73^{a}	56.71±0.55^{a}	67.64±0.47^{b}
	200	48.28 ± 0.46^{b}	48.64±0.69^{a}	61.51±0.49^{a}	69.58±0.34^{b}
	300	49.97 ± 0.50^{b}	57.61±0.53^{b}	67.45±0.48^{cd}	95.67±0.48^{f}
	400	53.11 ± 0.53^{c}	64.82±0.59^{c}	76.55±0.40^{f}	96.76±0.62^{f}
	500	54.76 ± 0.57^{c}	65.33±0.65c	72.51±0.49^{d}	100.±0.70^{g}
Methanol	100	54.20 ± 0.61^{c}	56.35±0.57^{b}	58.62±0.53^{a}	54.01±0.71^{a}
	200	49.78 ± 0.30^{b}	57.31±0.73^{b}	61.21±0.58^{ab}	55.51±0.50^{a}
	300	52.89±0.40^{c}	59.88±0.56^{bc}	62.62±0.23^{ab}	67.85±0.56^{b}
	400	54.65±0.09^{c}	62.37±0.51^{c}	64.22±0.62c	69.48±0.56^{b}
	500	56.34±0.16^{cd}	64.49±0.59^{c}	65.21±0.71^{c}	71.15±0.60^{bc}

*Value represents mean ± S.E. of five replication, each set-up with 20 adults. Values in the column with a different superscript alphabet are significantly different at *P < 0.05* level

Table: 2. Effect of Plant extract (*Mucuna pruriens*), Mean Mortality on adult weevils of *C.marculaltus**

Plant Name	**Mean mortality(±S.E) (%) at 1-4 days post treatment**				
Mucuna pruriens	**Conc.ppm**	**Day1**	**Day2**	**Day3**	**Day4**
Hexane	100	46.57±0.76^{c}	54.34±0.73^{b}	73.38±0.71e	85.65±0.62^{e}
	200	47.99±0.71^{c}	55.00±0.82^{b}	75.19±0.86e	97.87±0.85^{h}
	300	49.72±0.56^{c}	58.54±0.61^{c}	81.52±0.49^{f}	100.70±0.47^{h}
	400	52.06±0.68^{cd}	60.97±0.61^{d}	84.51±0.47^{f}	100.41±0.44^{h}
	500	56.00±0.77^{cd}	64.63±0.57^{b}	86.37±0.43^{g}	100.70±0.63^{h}
Diethyl ether	100	57.95±0.78^{d}	49.34±0.86^{a}	71.38±0.60^{e}	81.53±0.66^{d}
	200	60.24±0.87^{e}	51.64±0.56^{a}	63.50±0.59^{ab}	84.62±0.6^{e}
	300	42.32±0.76^{a}	53.22±0.82^{ab}	75.31±0.69^{e}	86.69±0.69^{e}
	400	44.65±0.84^{a}	55.54±0.68^{b}	77.29±0.46^{e}	93.77±0.60^{g}
	500	66.53±0.74^{f}	67.62±0.69^{f}	68.44±0.53^{d}	90.47±0.38^{f}
Dichloromethane	100	35.47±0.40^{a}	48.31±0.66^{a}	80.03±0.69^{f}	86.57±0.37^{e}
	200	48.76±0.61^{b}	49.61±0.53^{a}	74.56±0.67^{e}	89.65±0.41^{f}
	300	38.38±0.81^{a}	51.66±0.59^{a}	72.59±0.69^{e}	93.67±0.42^{g}
	400	52.86±0.81^{cd}	53.48±0.38^{ab}	81.80±0.56^{f}	97.39±0.49^{h}
	500	54.71±0.55^{c}	55.53±0.76^{ab}	86.62±0.68^{g}	98.38±0.48^{h}
Ethyl acetate	100	46.55±0.56^{b}	47.01±0.80^{a}	58.32±0.81^{a}	97.39±0.84^{h}
	200	47.67±0.31^{b}	49.41±0.56^{b}	60.18±0.81^{a}	98.26±0.70^{h}
	300	49.57±0.48^{b}	51.26±0.80^{a}	73.02±0.56^{e}	99.95±0.57^{h}
	400	52.48±0.79^{c}	53.86±0.51^{ab}	74.22±0.42^{e}	100.43±0.78^{h}
	500	52.85±0.91^{c}	65.65±0.66^{c}	87.57±0.47^{g}	100.92±0.77^{h}
Methanol	100	54.98±0.64^{c}	46.82±0.45^{a}	57.91±0.56^{a}	69.14±0.75^{a}
	200	56.02±0.64^{c}	57.69±0.71^{c}	59.58±0.48^{a}	69.58±0.54^{a}
	300	48.37±0.52^{c}	59.81±0.55^{c}	62.08±0.51^{ab}	72.66±0.50^{b}
	400	40.31±0.24^{b}	52.69±0.45^{a}	64.59±0.55^{c}	75.59±0.58^{c}
	500	43.07±0.76^{b}	55.15±0.65^{b}	66.77±0.55^{d}	77.42±0.53^{c}

*Value represents mean ± S.D. of five replications, each set-up with 20 adults. Values in the column with a different superscript alphabet are significantly different at *P < 0.05* level

Table: 3. Effect of plant extract (*Mucuna pruriens*) on grain damage

Name of the extract (MP)	con	Total no of Grains	Control	No of Perforated Grains	Unperforated Grains	% of Grain damage	WPI
Hexane	100	250	200	11	239	4.4	4.21
	200	250	200	13	237	5.2	4.94
	300	250	200	18	232	7.2	6.72
	400	250	200	26	224	10.4	9.42
	500	250	200	29	221	11.6	10.39
Diethyl ether	100	250	200	13	237	5.2	4.94
	200	250	200	15	235	6	5.66
	300	250	200	19	231	7.6	7.06
	400	250	200	24	226	9.6	8.76
	500	250	200	28	222	11.2	10.07
Dichloromethane	100	250	200	3	247	1.2	1.19
	200	250	200	7	243	2.8	2.72
	300	250	200	15	235	6	5.66
	400	250	200	19	231	7.6	7.06
	500	250	200	25	225	10	9.09
Ethyl acetate	100	250	200	8	242	3.2	3.10
	200	250	200	12	238	4.8	4.58
	300	250	200	17	233	6.8	6.37
	400	250	200	21	229	8.4	7.75
	500	250	200	31	219	6.4	6.02
Methanol	100	250	200	19	231	7.6	7.06
	200	250	200	15	235	6	5.66
	300	250	200	19	231	7.6	7.06
	400	250	200	26	224	10.4	9.42
	500	250	200	27	223	10.8	9.75

Weevil perforation Index (WPI)

Table: 4. Effect of plant extract (*Phaselous lunutus*) on grain damage

Name of the extract (PL)	Conc.	Total no of Grains	Control	No of Perforated Grains	Unperforated Grains	% of Grain damage	WPI
Hexane	100	200	150	0	200	0	4.21
	200	200	150	9	191	4.5	4.31
	300	200	150	14	186	7	6.54
	400	200	150	20	180	10	9.09
	500	200	150	22	178	11	9.91
Diethyl ether	100	200	150	9	191	4.5	4.31
	200	200	150	8	192	4	3.85
	300	200	150	12	188	6	5.66
	400	200	150	17	183	8.5	7.83
	500	200	150	20	180	10	9.09
Dichloromethane	100	200	150	1	199	0.5	0.50
	200	200	150	4	196	2	1.96
	300	200	150	11	189	5.5	5.21
	400	200	150	9	191	4.5	4.31
	500	200	150	18	182	9	8.26
Ethyl acetate	100	200	150	5	195	2.5	2.44
	200	200	150	9	191	4.5	4.31
	300	200	150	12	188	6	5.66
	400	200	150	18	182	9	8.26
	500	200	150	17	183	8.5	7.83
Methanol	100	200	150	15	185	7.5	6.98
	200	200	150	18	182	9	8.26
	300	200	150	22	178	11	9.91

	400	200	150	26	174	13	11.50
	500	200	150	29	171	14.5	12.66

Weevil perforation Index (WPI)

Table 1 presents the mean mortality (± standard error) of adult *Callosobruchus maculatus* weevils exposed to solvent extracts of *Phaseolus lunatus* seeds at concentrations of 100, 200, 300, 400, and 500 mg/L over a four-day period. The hexane extract showed strong insecticidal activity, with mortality increasing from 42.23% at 100 mg/L on Day 1 to 94.22% at 500 mg/L by Day 4. The dichloromethane extract demonstrated moderate effectiveness, with mortality rising from 49.14% at 100 mg/L on Day 1 to 97.07% at 500 mg/L on Day 4, indicating enhanced toxicity over time and concentration. Another extract also showed notable efficacy, starting at 54.61% mortality on Day 1 at 100 mg/L and reaching 100% by Day 4 at 500 mg/L, suggesting rapid and potent activity at higher doses. In contrast, the methanol extract exhibited the lowest toxicity, with minimal change in mortality—starting at 54.20% on Day 1 and slightly decreasing to 54.01% by Day 4—indicating limited effectiveness compared to the other extracts.

Table 2 presents result on the effectiveness of various *Mucuna pruriens* extracts against *C. marculatus* weevils at concentrations of 100 to 500 ppm over four days. This Hexane extract showed high mortality rates, starting at 46.57% on Day 1 at 100 ppm and reaching 85.65% by Day 4. Notably, 100% mortality was achieved at 300 ppm and higher, indicating hexane's strong insecticidal properties due to its ability to extract potent bioactive compounds [14]. Mortality was moderate compared to hexane, beginning at 57.95% at 100 ppm and peaking at 90.47% by Day 4 at 500 ppm. Its effectiveness improved with concentration, although it extracts insecticidal compounds less efficiently than hexane [15]. This Dichloromethane extract showed a steady increase in mortality, starting at 35.47% on Day 1 and reaching 98.38% by Day 4 at 500 ppm. This indicates effectiveness in controlling weevils, albeit at higher concentrations[16]. High efficacy was noted, with mortality starting at 46.55% and reaching 97.39% by Day 4. Full mortality was observed at 300 ppm and above, suggesting potent insecticidal properties that increase with concentration [17]. This Methanolic extract demonstrated the least effectiveness, starting at 54.98% mortality and only achieving 77.42% at 500 ppm. This suggests methanol's limitations in extracting potent insecticidal compounds[18] .

Table 3 shows the effects of different extracts from *Mucuna pruriens* on grain damage, concentrating on perforated grains, unperforated grains, the damage percentage, and the Weevil Perforation Index (WPI). The Hexane extract demonstrated notable grain damage at all concentrations, escalating from 4.4% at 100 ppm to 11.6% at 500 ppm, with a corresponding WPI increasing from 4.21 to 10.39, reflecting its ability to compromise grain integrity. The diethyl ether extract, comparable to hexane but less potent, resulted in 5.2% damage at 100 ppm and 11.2% at 500 ppm, yielding a WPI of 10.07, indicating a concentration-dependent effect. This dichloromethane extract exhibited slight damage at lower concentrations (1.2% at 100 ppm, WPI 1.19), which rose to 10% at 500 ppm, with a WPI of 9.09, reflecting enhanced potency yet still lower than hexane and diethyl ether. The ethyl acetate extract exhibited moderate harm (6.4% at 500 ppm, WPI 6.02) and reduced effect at 100 ppm (3.2%, WPI 3.10). The methanol extract showed comparable grain damage to hexane and diethyl ether (10.8% at 500 ppm, WPI 9.75) although it caused less damage at 100 and 200 ppm (7.6%).The findings show that the effectiveness of *Mucuna pruriens* extracts depends on the type and concentration of the solvent used. Hexane proved to be the most potent solvent, resulting in the greatest grain damage and the highest WPI, reflecting its powerful insecticidal capabilities. This aligns with earlier research indicating that non-polar solvents like hexane frequently extract a greater number of bioactive compounds with insecticidal properties [19].The extracts of diethyl ether and methanol also resulted in significant harm, but they were not as effective as hexane. Their growing efficacy with concentration indicates that their insecticidal agents exist but need greater concentrations to produce notable effects. Dichloromethane, although effective in higher concentrations, demonstrated the lowest activity at diminished levels, rendering it less efficient for protecting grains in comparison to other solvents. Ethyl acetate demonstrated moderate effects, akin to methanol, and might be suitable for uses requiring a gentler impact. Previous studies back these results, emphasizing the importance of solvent selection in obtaining the most potent compounds from plants, along with the concentration-related effects of plant-derived insecticides on grain damage[20].

Table 4 illustrates the impact of *Phaseolus lunatus* plant extracts on grain harm, assessed by the number of perforated grains, the percentage of grain damage, and the Weevil Perforation Index (WPI). Hexane extract led to an increase in grain damage from 0% at 100 ppm to 11% at 500 ppm, with the WPI growing from 4.21 to 9.91. The diethyl ether extract exhibited moderate effectiveness, with grain harm varying from 4.5% at 100 ppm to 10% at 500 ppm, along with a related rise in WPI from 4.31 to 9.09.The extract of dichloromethane resulted in slight damage at 100 ppm (0.5%) and rose to 9% at 500 ppm (WPI: 8.26). Ethyl acetate and methanol extracts showed moderate effectiveness, with damage rising from 2.5% at 100 ppm to 14.5% at 500 ppm, indicating a trend of improved grain protection with increasing concentration.The findings show that the hexane extract of *Phaseolus lunatus* was the most efficient in stopping grain damage, with notable rises in both grain damage and WPI at elevated concentrations. Diethyl ether and methanol demonstrated moderate insecticidal activity, whereas dichloromethane was generally less effective. These results align with earlier research, indicating that non-polar solvents such as hexane generally extract more effective insecticidal compounds from plants, providing enhanced pest protection [16]. However, while some plant powders exhibit insecticidal properties against pests like *C. maculatus*, others, like *M. tenuiflora* and *A. macrocarpa*, show limited effectiveness[21,22]. Factors affecting efficacy include the availability of strong plant materials and market challenges[23]. Although some plant powders demonstrate significant pest mortality, farmers suffer financial losses due to pest damage during storage, primarily due to insufficient pest management knowledge[24-26].

The research on extracts from *Mucuna pruriens* and *Phaseolus lunatus* showed different degrees of efficacy in managing grain damage inflicted by weevils. The hexane extract from *Mucuna pruriens* showed the greatest effectiveness, achieving notable grain protection at elevated concentrations (up to 11.6% damage at 500 ppm). Likewise, the hexane extract of *Phaseolus lunatus* demonstrated an escalation in grain damage from 0% to 11% with rising concentration. Diethyl ether and methanol extracts exhibited moderate efficacy, whereas dichloromethane had the least effect on grain damage. The Weevil Perforation Index (WPI) rose with concentration for many extracts, suggesting their promise as natural insecticides.

Conclusion

The study demonstrates that extracts from *Mucuna pruriens* and *Phaseolus lunatus*, especially those extracted with non-polar solvents like hexane, show promising potential as eco-friendly alternatives to chemical insecticides for managing *C. maculatus* infestations. Hexane extracts were the most effective in controlling invading weevil infestations and minimizing grain damage, though the seed extracts of methanol and dichloromethane extracts were shown some effect on arresting the weevil infestations but they are less effective. The results support the use of plant-derived insecticides for integrated pest management in grain storage, providing a safer and sustainable option to mitigate pest-related losses in agricultural products. Further research is needed to refine these methods and evaluate their long-term efficacy in real-world storage conditions. The exact mechanism of action of bio – control agents on pest infestation, the way forward.

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Declarations

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Conflicts of Interests

No conflicts of interest.

Data availability

All the data in this study is completely incorporated in the manuscript

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