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Research Article

Impact of *Achillea moschata* Wulfen on germination and growth of weed species *Echinochloa oryzoides* (Ard.) Fritsch and *Lolium multiflorum* Lam.

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Abstract

Weeding is a practice of primary importance both in conventional agriculture where the intensive use of herbicides can represent a significant environmental risk and in organic farming where this practice is prohibited. Therefore, the need to identify alternative means of controlling weeds is evident. In this respect, allelopathy proves to be a useful tool to be integrated with conventional agronomic techniques for the management of infesting flora. In this work, we investigated the phytotoxic potential of *Achillea moschata* Wulfen (Asteraceae) against *Echinochloa oryzoides* (Ard.) Fritsch and *Lolium multiflorum* Lam., two noxious plants for crops such as rice and wheat. Preliminary anti-germination assays were carried out in controlled conditions by testing both the powder (0.25 g) and the aqueous extract (1%, 10% and 20%) from the *A. moschata* aerial parts. The obtained results showed that the powder was more effective than the extract in inhibiting seed germination (up to –81.6% vs –48.8% at 20% concentration) and seedling development (up to –99.4% vs –75.9% at 20% concentration) of both target species, although *L. multiflorum* was more susceptible than *E. oryzoides* to treatments. Furthermore, the chemical composition of the two used matrices was characterized by Solid-Phase Microextraction (SPME) sampling technique and Gas Chromatograph/Mass spectrometer (GC/MS) analyses. Camphor (25.8% and 49.9%) and 1,8 cineole (25.9% and 20.7%) were the main constituents in the samples, followed by bornyl acetate (6.7%) in the powder and fragranol (10.5%) in the aqueous extract.

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1. Introduction

Weed management is of primary importance in conventional agriculture and, even more so, in organic agriculture. Due to various negative aspects for the environment and human health, the common importance of finding alternative strategies is evident. In this respect, allelopathy proves to be a useful tool to be integrated with conventional agronomic techniques. In recent years, especially in the international arena, there has been a growing interest methods of weed control by chemical means are increasingly accompanied by other agronomic practices capable of limiting the weed growth. In organic farming, where this practice is prohibited, the in the application of phytotoxic phenomena for the natural control of weeds. The plants releasing toxic compounds toward noxious species could allow to reduce the quantity of chemical herbicides, with an increase in both food and environmental safety [1].

At the moment, the agronomic affirmation of bioherbicides is limited by costs, but formulations that involve the use of substances able to enhance their toxic action are possible [2]. Therefore, it is of particular importance to test the effectiveness of extracts, essential oils or plant parts for the weed management in the agricultural ecosystem [3]. In this work, the inhibitory effects of *Achillea moschata* Wulfen (Asteraceae) were investigated through two different approaches, in order to evaluate its potential in relation to the containment of *Echinochloa oryzoides* (Ard.) Fritsch and *Lolium multiflorum* Lam., both belonging to the Poaceae family. *E. oryzoides* is a species adapting to different environments, from dry to humid ones, favored by periodic watering and, therefore, by a good presence of humidity. *E. oryzoides* is a common weed of rice fields, very aggressive and resistant, with a phenological cycle almost identical to that of the crop. Its marked ductility and great variability make it a difficult weed to fight even through the use of chemical herbicides [4]. *L. multiflorum* is a forage grass used as a cover crop, especially in organic farming, due to its characteristics of maintaining good fertility and soil structure and of controlling the adventitious flora. However, it is also a very competitive weed in agro-systems capable of causing crop yield losses (e.g., alfalfa, durum wheat and soft wheat) and for this reason regularly managed with chemicals such as glyphosate, against which it has shown resistance [5].

2. Materials and methods

2.1 Plant material

The seeds of *E. oryzoides* and *L. multiflorum* obtained from the organic farm Terre di Lomellina (Pavia, Italy) were selected and stored at 4 °C until use. The aerial parts of *A. moschata* were collected in full bloom in Valle dei Forni (Valfurva, Sondrio, Italy) at 2400 m above sea level after its identification according to Flora d'Italia [6]. A voucher specimen (No. AM-VDF-17) was deposited at the Department of Agricultural and Environmental Sciences, Milan State University, Italy.

2.2 Seed sterilization

The seeds of the two target species - *E. oryzoides* and *L. multiflorum*–were soaked in a solution of sodium hypochlorite (1%) and shaken for 10 minutes, then repeatedly rinsed with distilled water to remove the disinfectant.

2.3 A. moschata extraction

Dried and powdered aerial parts of *A. moschata* (10 g) were extracted by maceration (solid/liquid extraction) in distilled water (1:20) at room temperature for 24 h on a shaker, then filtered and centrifuged (3000 g/min, 30 min). Therefore, 3 different dilutions (1%, 10%, 20%) of the crude extract were prepared to be tested.

2.4 Anti-germination tests with A. moschata aqueous extract

Sowing in Petri dishes (90 mm) was carried out under a biological hood with vertical laminar flow. The seeds of each target species (10) were placed on two sheets of sterilized filter paper and subsequently soaked with 4 ml of extract. For each dilution (1%, 10%, 20%) of the extract, two runs with three replicates each were performed: *E. oryzoides* (N = 10x3x3x2) and *L. multiflorum* (N = 10x3x3x2). Control samples without *A. moschata* extract were also prepared. Subsequently, the initialed and sealed with Parafilm Petri dishes were incubated under controlled conditions (16 h of light at 23 °C and 8 h of dark at 18 °C) in a climatic chamber for 7 days and monitored every 24 h.

2.5 Anti-germination tests with A. moschata powder

Sowing in Petri dishes (90 mm) was carried out under a biological hood with vertical laminar flow. The seeds of each target species (10) were placed on two sheets of sterilized filter paper sprinkled with 0.25 g of coarsely chopped *A. moschata* and subsequently soaked with 5 ml of distilled water. Two runs with three replicates each were performed: *E. oryzoides* (N = 10x3x2) and *L. multiflorum* (N = 10x3x2). Control samples without *A. moschata* powder were also prepared. Subsequently, the initialed and sealed with Parafilm Petri dishes were incubated under controlled conditions (16 h of light at 23 °C and 8 h of dark at 18 °C) in a climatic chamber for 7 days and monitored every 24 h.

2.5 Seedling measurement

At the end of the tests, shoots and roots of *E. oryzoides* and *L. multiflorum* were measured by observation

under a stereomicroscope to verify the phytotoxic impact of *A. moschata* on their germination and growth. Then, some indices were calculated:

1) Germination percentage (G) = Germinated seed number)/(Seed total number) × 100;
2) Coefficient of Velocity of Germination (CVG) = N1 + N2 + . . . + Ni/100 × N1T1 + . . . + NiTi, where N is the number of seeds germinated every day; T is the number of days from seeding corresponding to N [7];
3) Mean Germination Time (MGT) = (∑D × Germinated seed number)/(∑Germinated Seed number), where D is the number of days from the beginning of germination, plus the number of seeds germinated on day D [8];
4) Seedling Vigor Index (SVI) = (Mean Root length + Mean Shoot length) × Germination % [9].

2.6 SPME-GC-MS analyses

2.6.1 *SPME sampling*

To sample the volatile fraction of the aqueous extract and the powder of *A. moschata*, we used a SPME device from Supelco (Bellefonte, PA) 311 with 1 cm fiber coated with 50/30µm DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane).
Approximately 2 g of powder and 2 mL of crude extract (100%) were placed inside a 15 mL and 4 mL glass vial with PTFE-coated silicone septum, respectively. The optimized applied parameters were as previously reported [5], with some minor modifications such as temperature (55 °C) and heating time (40 min).

2.6.2 *GC-MS*

To investigate the volatile chemical profile of both samples, a gas chromatograph coupled with a mass spectrometer (Clarus 500 model Perkin Elmer, Waltham, MA, USA) and equipped with a FID (flame detector ionization) was used. The oven GC was equipped with a Varian Factor Four VF-1 capillary column (60 m length x 0.32 mm ID and 1.0 µm film thickness). The operative GC-MS conditions following Garzoli et al. (2022) [10].
The identification of the separated components was carried out first by matching their mass spectra with those stored in the Wiley 2.2 and NIST 02 mass spectra libraries database and then by calculation of the Linear Retention Indices (LRIs) relative to C_8–C_{30} aliphatic hydrocarbons, injected at the same operating conditions and compared with available retention data in the literature. The quantification of the compounds, obtained from the peak areas of the FID signal, was expressed as relative percentages. The analyses were carried out in triplicate.

2.7 Statistical Analysis

The data were evaluated with the support of IBM SPSS software, through the analysis of variance carried out separately for each *A. moschata* tests (i.e., aqueous extract and powder). The germination and growth indices (i.e., G%, CVG, MGT, SVI, root length, shoot length) measured for the two target species (i.e., *L. multiflorum* and *E. oryzoides*) under different treatments were considered as dependent variables.

The one-way ANOVA and the Turkey's-b post hoc test were performed in order to establish the significant effect (at $p \leq 0.05$) of the treatments with *A. moschata* tests (i.e., the different levels of concentration or quantity in extract and powder, respectively) on the target species and describe the homogenous subsets. Post-hoc test was not performed if fewer than three groups were present (*A. moschata* powder test). Moreover, the two-way ANOVA was performed, considering as factors the treatments with *A. moschata* extract and powder over the species, in order to highlight the significant interaction ($p \leq 0.05$) between "species × treatments" and then highlighting the species-specific effects of the treatments and the different behavior or susceptibility of *L. multiflorum* and *E. oryzoides*.

3. Results

3.1 A. moschata phytotoxicity

In general, the data reported in tables 1 and 2 show a greater efficacy of the *A. moschata* powdered aerial parts compared to their aqueous extract in inhibiting the seed germination and the seedling development of both target species. Of these, *L. multiflorum* was found to be more susceptible to *E. oryzoides*.

The phytotoxic effects of *A. moschata* powder are shown in table 1. The germination rate of *E. oryzoides* and *L. multiflorum* decreased by 29.7% and 81.6%, respectively. Consequently, their calculated indices were significantly reduced with respect to the controls. In detail, CVG of *E. oryzoides* decreased by 60.1% while that of *L. multiflorum* by 87.5%. Their SVIs dropped to −80.7% and −99.4% impacted by stunted growth of both root (−83.8% and −98%) and shoot (−50% and −94.7%). MGT values increased by 7.4% and 41.7%, respectively.

The aqueous extract at the lowest concentrations (1% and 10%) was not effective in significantly reducing the percentage of seed germination of the two weeds. Otherwise, when used at the highest concentration (20%) (Table 2), it was able to limit the germination of *E. oryzoides* to 76.7% and to halve that of *L. multiflorum* (−48.8%). This, together with the considerable reduction in root (−54.2% and −66.3) and shoot (−15.4% and −37.8%) development, resulted in significantly lower CVG (−38.1% and −71.1%) and SVI (−55.7 and −75.9%) values than in controls. MGT increased up to 7.5% for *E. oryzoides* and up to 14% for *L. multiflorum*.

3.2 Chemical analysis

The aqueous extract and the powder obtained from the aerial parts of *A. moschata* and analyzed by SPME-GC-MS technique showed a similar volatile chemical composition, albeit with both qualitative and quantitative differences (Table 3). In detail, camphor (49.9% and 25.8%) and 1,8-cineole (20.7% and 25.9%), present in similar percentages in the powder, were the principal components in the extract and in the powder, respectively, although with an opposite trend. As expected, more components were detected in the powder [22] than in the extract [8]. Among these, the sesquiterpene compounds such as *β*-caryophyllene, humulene and sesquicineole were completely absent in the aqueous extract. Other compounds, belonging to the monoterpene family and ranging from 0.5 to 5.9%, were detected in the powder and not in the extract. The chromatograms were reported (Figure 1).

4. Discussion

The bioassays were set up in order to evaluate the phytotoxic effect of *A. moschata* against two monocot weeds. Although the aqueous extract was less active than the powder, its inhibitory activity (at 10% and 20% concentrations) on root growth both of *E. oryzoides* and *L. multiflorum* were evident. Roots were less developed but with denser hairs than the controls, confirming themselves as the most sensitive vegetative structures to treatments [11].

Table 1. Germination and growth parameters of two target species *E. oryzoides* and *L. multiflorum* under the phytotoxic effects of *A. moschata* powder.

Target species	*A. moschata* powder (g)	G (%)	CVG	MGT	SVI	Root (mm)	Shoot (mm)
E. oryzoides	0	90.0±8.9	41.1±6.0	5.4±0.2	8131±1054	59.9±3.2	30.2±3.3
	0.25	63.3±10.3	16.4±6.5	5.8±0.3	1572±302	9.7±0.9	15.1±2.5
	F	22.857	50.251	9.988	214.698	1386.754	81.544
	p-value	0.001*	0.000*	0.010*	0.000*	0.000*	0.000*
L. multiflorum	0	81.7±11.7	51.6±9.9	4.8±0.1	9768±1906	65.7±5.5	53.3±3.9
	0.25	15.0±5.5	0.7±0.6	6.8±0.3	61±25	1.3±0.3	2.8±0.8
	F	160.000	162.667	338.780	155.660	815.503	967.112
	p-value	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
Interaction species x treatment							
	F	27.170	24.446	64.286	12.296	29.268	228.502
	p-value	0.000*	0.000*	0.000*	0.002*	0.000*	0.000*

Values are mean ± standard deviation; asterisk indicates statistically significant differences at p-value ≤ 0.05 among treatments in each species. F-value and p-value of the ANOVA test. Abbreviations: G%, Germination percentage; CVG, Coefficient of Velocity of Germination; MGT, Mean Germination Time, SVI, Seedling Vigor Index.

Table 2. Germination and growth parameters of the two target species *E. oryzoides* and *L. multiflorum* under the phytotoxic effects of different concentrations of *A. moschata* extract.

Target species	*A. moschata* extract (%)	G (%)	CVG	MGT	SVI	Root (mm)	Shoot (mm)
E. oryzoides	0	100±0.0^{a}	49.4±3.1^{a}	5.3±0.1^{a}	9520±537^{a}	65.9±6.3^{a}	29.3±2.9 ^{a}
	1	93.3±5.2^{ab}	43.1±3.2^{b}	5.4±0.1^{ab}	8767±767^{a}	65.4±5.2^{a}	28.5±2.0^{a}
	10	90.0±0.0^{b}	33.3±5.2^{c}	5.7±0.3^{c}	5831±218^{b}	38.1±1.7^{b}	26.7±1.6^{ab}
	20	76.7±10.3^{c}	30.6±5.1^{c}	5.6±0.2^{bc}	4222±64^{c}	30.2±3.2^{c}	24.8±2.3^{b}
	F	1.333	24.876	6.267	110.693	103.375	4.775
	p-value	0.000*	0.000*	0.004*	0.000*	0.000*	0.011*
L. multiflorum	0	71.7±7.5^{a}	35.9±4.3^{a}	5.0±0.1^{a}	7402±612^{a}	55.8±3.2^{a}	47.9±3.8^{a}
	1	65.0±5.5^{ab}	31.5±5.9^{a}	5.1±0.0^{a}	6395±400^{b}	54.6±2.6^{a}	44.0±3.1^{ab}
	10	58.3±4.1^{b}	23.1±3.6^{b}	5.3±0.2^{a}	4490±488^{c}	36.9±2.9^{b}	39.9±2.7^{b}
	20	36.7±5.2 c	10.4±4.3^{c}	5.7±0.4^{b}	1785±399^{d}	18.8±1.9^{c}	29.8±5.9^{c}
	F	42.521	36.087	10.794	151.150	250.270	21.702
	p-value	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
Interaction species x treatment							
	F	2.764	2.954	3.512	2.678	5.117	9.313
	p-value	0.054	0.044*	0.024*	0.060	0.004*	0.000*

Values are mean ± standard deviation; asterisk and different letters indicate statistically significant differences at *p*-value ≤ 0.05 among treatments in each species. *F*-value and *p*-value of the ANOVA test. Abbreviations: G%, Germination percentage; CVG, Coefficient of Velocity of Germination; MGT, Mean Germination Time, SVI, Seedling Vigor Index.

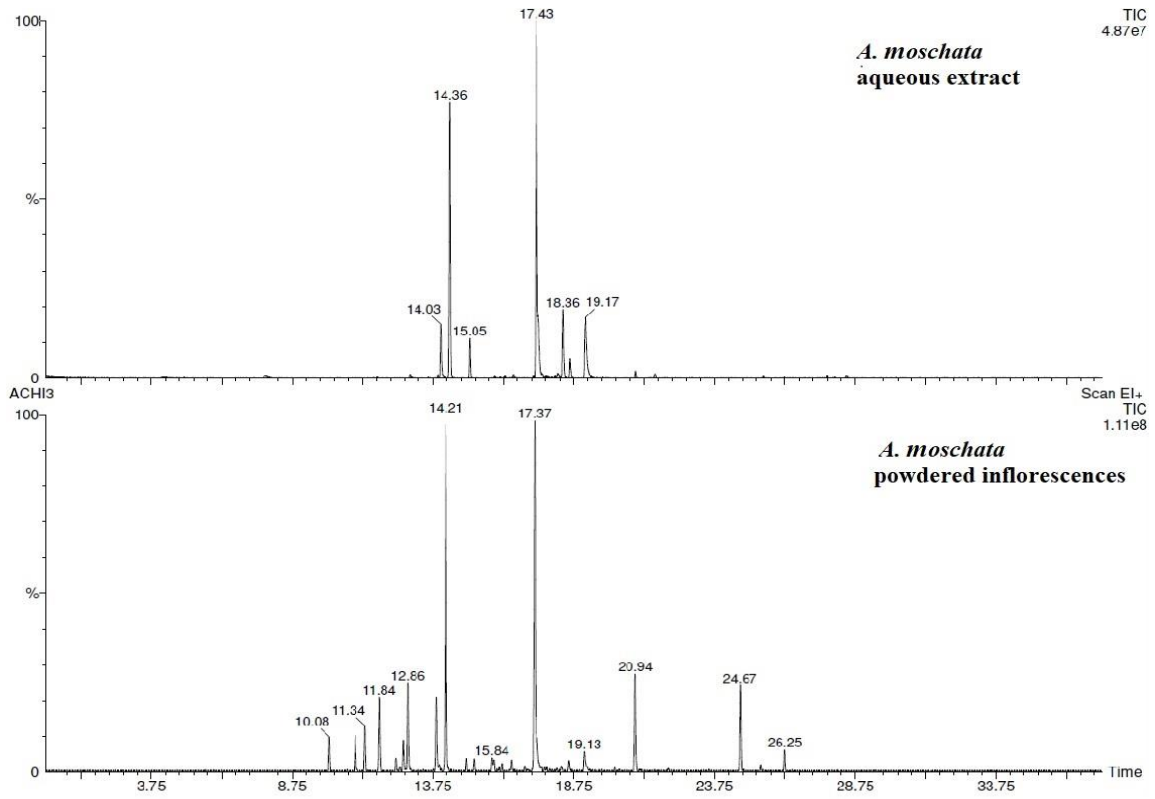

Figure 1. Chromatograms of *A. moschata* powdered aerial parts and their aqueous extract.

Table 3. Volatile chemical composition of *A. moschata* powdered aerial parts and their aqueous extract.

Compounds[1]	**Class**	**LRI**[2]	**LRI** [3]	***A. moschata* powder** [4]**(%)**	***A. moschata* aqueous extract** [5]**(%)**
Santolina triene	MH	903	905,3	2.2±0.02	-
α-Thujene	MH	921	923	2.6±0.03	-
α-Pinene	MH	948	945	3.0±0.03	-
Camphene	MH	942	948	5.3±0.03	-
β-Pinene	MH	980	986	0.9±0.03	-
α-Terpinene	MH	1010	1010	1.9±0.05	-
p-Cymene	MH	1012	1016	5.2±0.06	5.6±0.04
1,8-Cineole	OM	1027	1025	25.9±0.07	20.7±0.02
Cis-β-ocimene	MH	1030	1033	5.7±0.03	-
γ-Terpinene	MH	1057	1054	0.8±0.02	3.4±0.03
Terpinolene	MH	1079	1080	0.8±0.03	-
Iso-amyl, 2-methyl butyrate	FA	1090	1094	0.9±0.04	-
Chrysanthenone	OM	1101	1103	0.5±0.04	-
Isovaleric acid, 2-methylbutyl ester	FA	1109	1107	0.8±0.02	-
Camphor	OM	1130	1125	25.8±0.04	49.9±0.05
Trans-sabinene hydrate	OM	1145	1140	0.4±0.03	7.5±0.02
α-Terpineol	OM	1185	1182	0.9±0.02	2.0±0.02
Fragranol	OM	1224	1220	2.2±0.02	10.5±0.03
Bornyl acetate	OM	1268	1262	6.6±0.03	0.4±0.02
β-Caryophyllene	SH	1422	1424	5.9±0.02	-
Humulene	SH	1470	1473	0.4±0.02	-
Sesquicineole	OS	1501	1503	1.3±0.02	-
Number of identified compounds				**22**	**8**
TOTAL				100.0	100.0
Monoterpene Hydrocarbons (MH)				28.4	9.0
Oxygetaned Monoterpenes (OM)				62.3	91.0
Sesquiterpe Hydrocarbons (SH)				6.3	-
Oxygenated sesquiterpenes (OS)				1.3	-
Fatty acids (FA)				1.7	-

[1]The components are reported according to their elution order on apolar column; [2]Linear Retention indices measured on apolar column; [3] Linear Retention indices from literature; *: LRI not available [4] Percentage mean values ± SD (standard deviation) of powdered inflorescences *A. moschata* components; [5] Percentage mean values ± SD (standard deviation) of aqueous extract of *A. moschata* components; - Not detected.

Previously, other *Achillea* species showed phytotoxic activity. For instance, Polatoğlu and co-authors [12] investigated the essential oils of *A. biebersteinii* Afan., *A. vermicularis* Trin. and *A. teretifolia* Willd. finding them promising as potential bioherbicides. Similarly, Çakır and collaborators [13] documented the herbicidal properties of *A. biserrata* Bieb., *A. wilhelmsii* C. Koch, *A. biebersteinii* and *A. coarctata* Poir. against some dicot weeds by testing their essential oils as well as *n*-hexane, acetone and methanol extracts. Recently, Elshamy et al. [14] demonstrated the greater activity of the *A. fragrantissima* (Forssk.) Sch.Bip. essential oil extracted by microwave compared to that obtained by hydrodistillation on germination, root and shoot growth of *Lactuca sativa* L. Some investigations carried out on the aqueous extracts of *A. santolina* L. showed its ability to induce allelopathic effects towards different species including weeds and crops [15-18].

With the application of the SPME-GC-MS technique, we have analyzed for the first time the volatile composition of *A. moschata* extract and powder. No

other similar work is present in literature. Some authors have reported the chemical content of the essential oil and of various extracts obtained from aerial parts of *A. moschata* [19-21] or other *Achillea* species such as *A. millefolium* L., *A. ligustica* All., *A. clavennae* L., *A. coarctata* Poir., *A. kotschyi* Boiss., *A. monocephala* Boiss. & Balansa, *A. pachycephala* Rech.f. and *A. alpina* L. [22-29] highlighting the presence of several secondary metabolites by analysis with different techniques including NMR, HPLC-MS, LC-MS-MS and LC/IT-TOF-MS. The results obtained with our study showed a volatile fraction of *A. moschata* rich in monoterpenes and terpene derivatives with camphor and 1,8-cineole as main constituents. Together with other monoterpenes, they are already known to be allelochemical compounds [30]. For example, Okamoto et al. [31] reported that the camphor released from the *Cinnamomum camphora* (L.) J.Presl leaf powder in the soil and absorbed from the air through the stomata was the main responsible for the phytotoxic activity detected against the growth of *Oryza sativa* L. used as a receiver plant. The results obtained by Nishida et al. [32] suggested that both camphor and 1,8-cineole could interfere with the growth of other plants by inhibiting the cell proliferation in the root apical meristem.

5. Conclusions

Although the data are preliminary, we can conclude that *A. moschata* synthesizes phytotoxic compounds capable of influencing the growth of neighboring plants. Future studies could aim to deepen the evaluation of its herbicidal potential through the use of both higher extract concentrations and quantities of powder. Further investigation phases will be able to complete the characterization of the aqueous extract in relation to its non-volatile fraction and, therefore, provide useful information on the mechanism of action responsible of the observed activity.

Author Contributions:

Conceptualization, S.V. and M.I.; Methodology, S.V., S.G., M.I.; Validation, S.V., S.G. and V.V.; Formal Analysis, V.V.; Investigation, S.V., S.G.; Resources, S.G., M.I.; Data Curation, S.V., S.G.; Writing – Original Draft Preparation, S.V., S.G., V.V.; Writing – Review & Editing, S.V., M.I.; Supervision, M.I.

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Conflicts of Interest:

The authors declare no conflict of interest.

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