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Field assessment of *Phytomyza orobanchia* Kalt. (Diptera, Agromyzidae) as a natural enemy of the parasitic weed *Orobanche crenata* Forsk. (Orobanchaceae)

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Abstract

Background The broomrape, *Orobanche crenata* Forsk. (Orobanchaceae) is a noxious root parasite weed which seriously damages the productivity of many crops, particularly in the Legumes such as faba bean *Vicia faba*. The parasitism caused by this weed leads to significant yield losses in infested fields.

Results This study investigated the natural occurrence and infestation dynamics of the fly, *Phytomyza orobanchia* Kalt. in faba bean fields naturally infested by the parasitic weed *O. crenata*. The study was conducted during three successive faba bean seasons, 2020–2023 during, February, March, and April in two locations; Abies and Nubaria farms. The fly populations were generally low in the three seasons in February, increase to its maximum in late March in the 1st season and in April during the 2nd and 3rd seasons. In Nubaria farm, most larvae and puparia of *Ph. orobanchia* fly were found within the seed capsules of the *O. crenata*, with significantly lower proportions in plant base and stem parts. The fly preferred to attack the middle and upper fruits than lower ones. In contrast, at Abies farm, most of *Ph. orobanchia* flies were significantly found at plant base followed by seed capsules, and the lowest proportion was in the plant stem.

Conclusions Infestation with *Ph. orobanchia* fly is efficient in reducing *Orobanche* seed yield so it has a great effect in the reduction of *Orobanche* seed bank. The findings suggested that the fly, *Ph. orobanchia* could serve as a promising biological control agent for *Orobanche* spp. in Egypt, especially when combined with cultivation of resistance faba bean cultivars and the application of mass rearing and release program in infested areas.

Keywords *Orobanche crenata*, *Phytomyza orobanchia*, *Vicia faba*, Biological control, Egypt

Background

The parasitic weed broomrape, *Orobanche crenata* Forsk. (Orobanchaceae) is a highly destructive root parasite which seriously constrains the productivity of many crops, belonging to the Leguminosae and Solanaceae families in Egypt (Parker and Riches 1993; Hegazi et al. 2024a). Various control methods have been tested for broomrape control, but none has proved entirely satisfactory (Saghir 1979; Saghir et al. 1980; Linke

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and Saxena 1991). The main challenges arise from the weed's production of a large number of seeds ranging from 30,000 to 200,000 per plant (Schroeder 1994), which can remain viable in the soil for several years. Additionally, the intimate physiological interaction between *Orobancha* and its host plants limits the application of conventional weed control measures largely ineffective.

Several records show that biological control of *Orobancha* has been successfully achieved in the field. The fly *Phytomyza orobanchia* Kalt. (Diptera: Agromyzidae) is widely distributed occurs in Egypt, but it was too often killed by routine use of insecticides (Fessehaie and Parker 1992; Hegazi et al. 2024a, b).

Successful biological suppression of *Orobancha* spp. using *Ph. orobanchia* has been documented in several countries, the former USSR (Klyueva and Pamukchi 1983; Horvath and Wittmann 1988), Turkey (Giray and Nemli 1983), Syria (Linke et al. 1990; Linke 1992), and in India (Manjunath and Nagurkatti 1977).

Broomrape fly, *Ph. orobanchia* is oligophagous feeding only on *Orobancha* species. It feeds mainly on the immature seeds destroying between 11 and 90% of seeds (Sauerborn 1991). Also, it causes reduction in the length of shoots and number of capsules/shoot. One larva can destroy all seeds in the small capsule of broomrape, but the big capsule needs more than one larva for complete reduction of seeds. The natural potential of *Ph. orobanchia* to reduce the *Orobancha* population is mainly limited by low temperatures, cultural practices (soil preparation, crop rotation, irrigation, and the use of insecticides against crop pests) and natural enemies (microorganisms, parasitoids, and predators). The aim of the present study was to determine the efficiency of *Ph. orobanchia* as a biocontrol against *O. crenata* in faba bean (*Vicia faba* L.) fields in Alexandria, Egypt.

Methods

Study sites and plant material

The occurrence of *Ph. orobanchia* fly was investigated in faba bean fields naturally infested by the parasitic weed *O. crenata*, in two locations in Egypt; namely, Abies (31°12' 28" N, 29°58' 10" E) and Nubaria (31°04' 08" N, 29° 49' 25" E) farms. The study was conducted during three successive faba bean seasons, 2020–2023 (February–April). In both locations faba bean cultivar, Giza 843 was cultivated. The cultivar is known for its resistance to *O. crenata* infestation. The recommended agricultural practices for faba bean were applied. Notably, no chemical fertilizers or pesticides treatments were administered throughout the duration of the seasons.

Sampling and insect identification

In each visit, thirty broomrape infested faba bean plants were randomly sampled per replicates, with three replicates per experimental treatment. Samples were gently uprooted for investigation. Identification of *Ph. orobanchia* fly was carried out following Spencer (1973). All data of the present work were from broomrape plants at full flowering to early ripening stage. All *O. crenata* shoots were collected randomly per Expt., with at least 2 m between samples by walking diagonally across the fields and along the peripheries (Abbasher 1994).

Natural incidence and infestation rates with agromyzid fly, *Ph. orobanchia*

Variations of population dynamics of *Ph. orobanchia* during the three successive faba bean seasons (2020–2023) cultivated in Nubaria area 45 km west Alexandria were studied. Samples of *O. crenata* shoots at full flowering to early ripening stage were successively weekly collected from faba bean farms in the area and dissected. The number of flies in different parts of the spike (= capsules, stem, and tubercle) was recorded during February, March, and April (cool to warm temperature).

Distribution pattern of *Ph. orobanchia* fly in *O. crenata* spikes

In April of the first season, new samples of broomrapes were examined in faba bean farms of two locations; Abies and Nubaria where the fruit capsules, stems, and plant base of parasitic weed were dissected, and number of *Ph. orobanchia* flies in each was recorded.

In Nubaria farm, another set of parasitic weed of well-developed and almost of equal lengths were collected to study the effect fruit capsule level on infestation rate by *Ph. orobanchia* flies. All fruit capsules were detached from upper, middle, and lower levels of the parasitic plant and checked for fly infestation, where percentage of insect infestation in fruits of each level was recorded.

Assessment of *Orobancha crenata* seed yield and reduction due to *Ph. orobanchia* infestation

Sets of well-developed *O. crenata* spikes were collected from Nubaria faba bean farm in 2023 season to study the *O. crenata* seed yield and its reduction due to *Ph. orobanchia* parasitism. All capsules of the plant samples were separated and counted. Capsules were then air-dried. The fruit capsules were dissected categorized based on infestation level: uninfested (healthy), infested with one pupa, two pupae, or three or more pupae per capsule. The ripe seeds from each group were weighed using a microbalance of high accuracy (± 0.01 mg; Sartorius AG, Goettingen, Germany), and the seed yield per

capsule category was calculated to estimate the reduction attributable to fly infestation. The voucher specimens were deposited in Entomology Department.

Climate in the farms

The daytime temperatures in February range from 17 to 22 °C, while nighttime temperatures can drop to 8 to 12 °C. Rainfall is generally minimal, though occasional temperatures typically range from 18 to 25 °C, while nighttime temperatures may shower particularly in Alexandria. The temperature in March gradually warm up. Daytime vary from 10 to 15 °C remains relatively low, though occasional showers are possible, especially in coastal areas. In April, temperatures start to rise, with daytime highs ranging from 20 to 30 °C. Nighttime temperatures are cooler, typically between 12 and 18 °C. April is generally dry, with minimal rainfall across most sites.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) where appropriate to determine differences between means. Data of seeds weights were subjected statistically using T-test ($p > 0.05$). Data are presented as means of number $\pm$ SE.

Results

Occurrence of *Phytomyza orobanchia* in faba bean fields

The soil of faba bean farms was heavily infested with *O. crenata*. Some of the infested faba bean plants by *O. crenata* in locations near sites treated with insecticides were infestation *Ph. orobanchia* free. Weekly variations of population dynamics of *Ph. orobanchia* on *O. crenata* spikes during three successive faba bean seasons (2020–2023) cultivated in Nubaria area 45 k West Alexandria are tabulated in Table 1. The data in Table 1 indicated that the mean number of *Ph. orobanchia* on *O. crenata* spikes in the 1st season (2020–2021) was low during the 1st week of February 6 (14.24 ± 1.12 /spike) reaching its significant peak ($F=51.5$, $df=7,16$, $P<0.05$) on March 21 (42.16 ± 1.95 /spike). On the 2nd and 3rd seasons, the fly populations were generally lower than in the 1st season. The mean number of fly population was low in February 6 (4.46 ± 0.86 /spike) increased significantly ($F=51.5$, $df=7,16$, $P<0.05$) in April 3 (17.7 ± 1.55 /spike), in the second season. In the 3rd season, the population was also low in February 15 (10.17 ± 0.00 /spike), significantly increased ($F=28.73$, $df=7,16$, $P<0.05$) in April 7 (26.78 ± 1.00 /spike). The occurrence of *Ph. orobanchia* flies on *O. crenata* spikes shows in Table 1. The fly population was generally low in the three seasons in February, but increase to its maximum in late March in the 1st season and April 3 in the 2nd and April 7 in the 3rd seasons.

Table 1 *Ph. orobanchia* fly population dynamics during three faba bean seasons cultivated in Nubaria 45 km far from Alexandria

Sampling date	Season		
	2020–2021	2021–2022	2022–2023
Total insect no./plant			
6-Feb	$14.24^e \pm 1.12$	$4.46^d \pm 0.86$	$13.7^c \pm 0.23$
15-Feb	$16.29^e \pm 1.17$	$6.56^d \pm 0.96$	$10.17^d \pm 0.00$
22-Feb	$21.08^d \pm 1.53$	$8.75^d \pm 1.65$	$13.52^c \pm 0.13$
9-Mar	$28.25^c \pm 2.46$	$11.33^c \pm 0.6$	$14.81^c \pm 0.55$
16-Mar	$35.21^b \pm 1.27$	$13.73^b \pm 0.7$	$15.64^c \pm 0.60$
21-Mar	$42.16^a \pm 1.95$	$16.12^a \pm 0.8$	$18.15^b \pm 0.93$
3-Apr	$19.68^d \pm 0.92$	$17.7^a \pm 1.55$	$17.26^c \pm 4.19$
7-Apr	$17.91^d \pm 0.48$	$8.61^d \pm 0.42$	$26.78^a \pm 1.00$

Up to 42.2, 17.7, and 26.8 larvae and pupae have been counted in one shoot of these dates, respectively. So, the population of the fly was different ($F=7.6$, $df=2,21$, $P<0.05$) from year to year.

When the letter per each column is the same, the data for separately season are not significantly different ($P<0.05$).

Distribution pattern of *Ph. orobanchia* fly in *O. crenata* spikes

The eggs of *Ph. orobanchia* fly are deposited into fruit capsules or under the epidermis of the shoots. The larvae feed on the immature seeds and subepidermal tissue, sometimes penetrating into the shoots where they feed. Pupation takes place in the capsules but some in the stem or deeper in the hidden part of the stem base near the ground. In the 1st season (2020–2021), the distribution pattern of *Ph. orobanchia* fly in different parts of *O. crenata* spikes was studied at the same time (April) in the two locations of faba bean farms, namely, Abies and Nubaria farms (Fig. 1). The results revealed that differences between the investigated fields were significant ($F=7.6$, $df=2,21$, $P<0.05$). The distance between the two farms was 45 km; however, in Nubaria farm, most larvae and puparia of *Ph. orobanchia* fly were found in fruits capsules with significantly lower proportions in plant base and stem parts ($F=6703.96$, $df=2,6$, $P<0.05$). While in Abies farm, most of *Ph. orobanchia* flies were significantly found in plant base followed by fruit capsules, and the lowest proportion was in the plant stem ($F=998.9$, $df=2,6$, $P<0.05$).

In Nubaria farm, in 2020–2021 season, the fruit capsules of *O. crenata* spikes samples were divided into three levels: upper, middle, and lower ones to study the influence of capsule level on fly infestation rate. Fruits

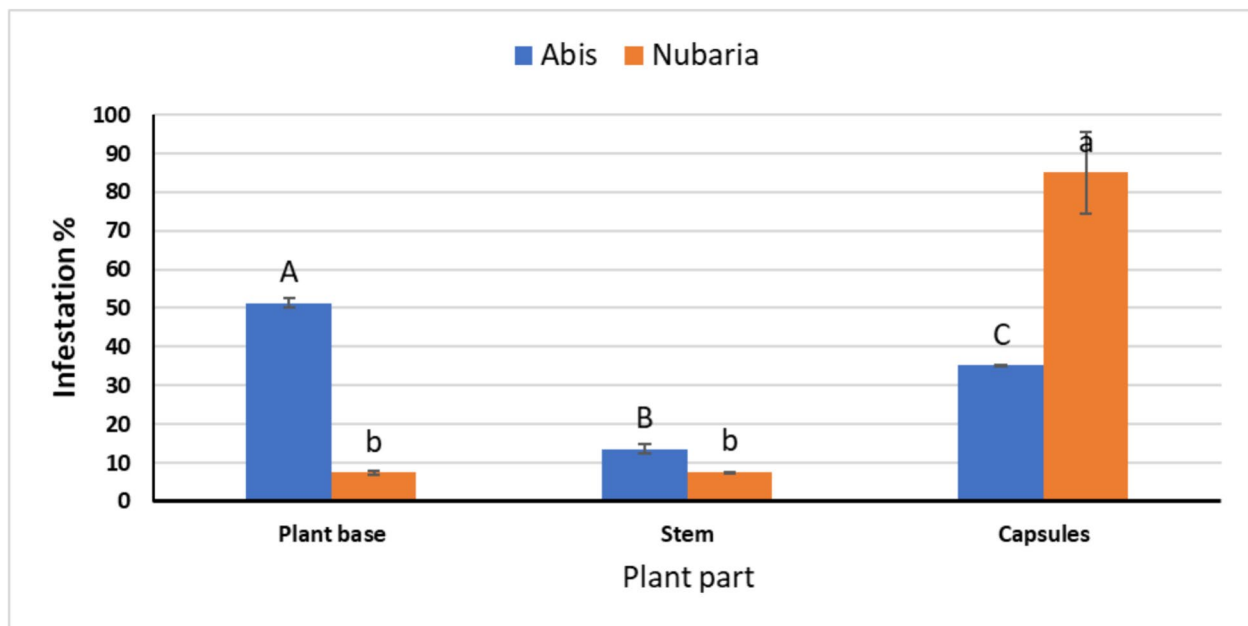


Fig. 1 Distribution pattern of *Ph. orobanchia* on different parts of infested *O. crenata*, in 2020–2021 season (Abies and Nubaria farms). For each farm, bars with the same letter are not significantly different ($P < 0.05$)

of each part of plant spike were examined for the presence of *Ph. orobanchia* flies (Fig. 2). Infestation rates by the fly among these groups of fruits were significant ($F = 18.41$, $df = 2, 6$, $P < 0.05$). It seems that the fly prefers to attack the middle and upper fruits than lower ones.

Effect of infestation by *Ph. orobanchia* on *Orobancha crenata* seed yield

As shown in Table 2, two sizes of *O. crenata* fruit capsules were observed, small weighed 31.3 ± 2.97 mg and large ones weighed 94.9 ± 6.4 mg. The difference was significant ($t = 31.3$, $P < 0.05$). The ratio between the two sizes was almost 1:1. The average number of ripe seeds

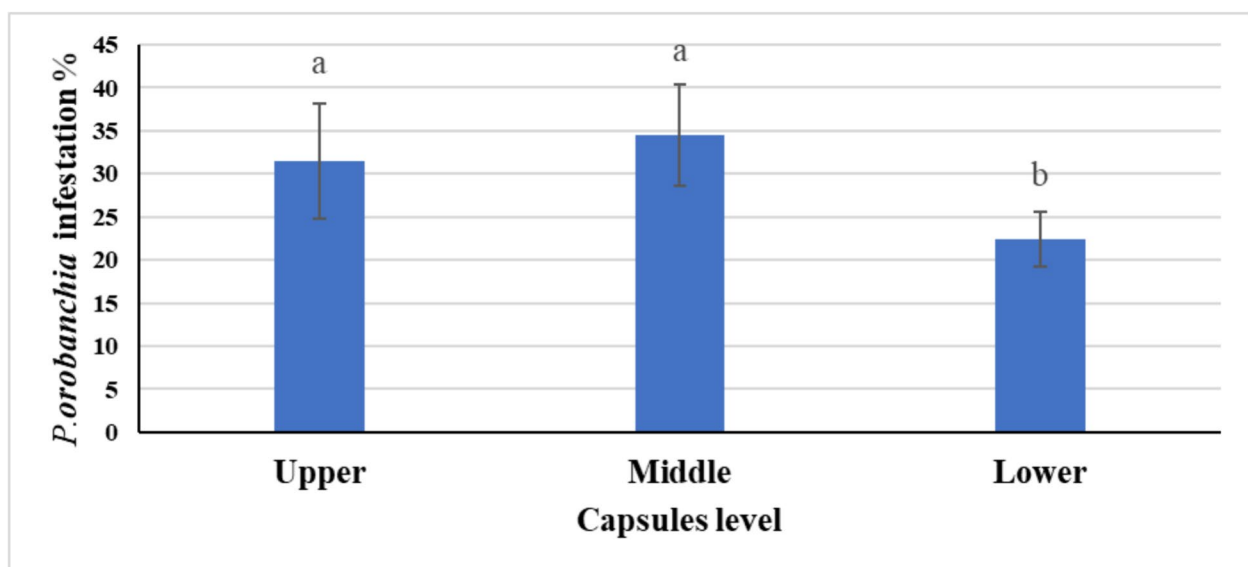


Fig. 2 Influence of capsule level on infestation rate by *Ph. orobanchia* (2020–2021 season) in Nubaria farm. Bars with the same letter are not significantly different ($P < 0.05$)

Table 2 Weight (mg) and seed number in healthy air-dried capsule of *O. crenata* capsules

Capsule		Capsule weight (mg)	Ripe seeds/capsule	
			Weight (mg)	Number
Small	Range	19.7–54.1	7.1–26.3	1347.5–4991.3
	Mean	31.3 ± 2.97	13.9 ± 1.8	2484.2 ± 341.8
Large	Range	61.7–130.1	23.6–50.7	4478.9–9622.1
	Mean	94.9 ± 6.4	36.6 ± 2.6	6958.7 ± 493
Overall mean		63.1 ± 8.1	24.85 ± 3.1	4721.49 ± 590.48

found/capsule was 2484.2 ± 341.8 and 6958.7 ± 493 of tiny seeds in small and large fruit capsules, respectively. The larvae of *Ph. orobanchia* mine the fruit capsules and feed on the immature seeds. Shrinkage and rotting of the fruit capsules suggest symptoms of fly infestation. Dissecting these fruits will show complete destruction of some or all seeds and accumulation of larval feces. One seed capsule is generally sufficient for six larvae to develop to pupation. However, *Ph. orobanchia* fly infestation reduced *O. crenata* seeds by destroying 59.6, 91.36, and 100% of the seed capsules (Table 3) when the capsule attacked by 1, 2, or 3 of *Ph. orobanchia* larvae, respectively. The weight of ripe seeds (size 0.3×0.2 mm) in the healthy capsule and those infested by 1, 2, and 3 fly larvae was 24.85 ± 3.1 , 8.85 ± 2.6 , 2.15 ± 0.9 and 0.0 mg, respectively. The difference was significant ($F=1347.1$, $df=3, 36$, $P<0.05$). In Nubaria, the flowering broomrape shoot ($n=30 \times 3$ reps.) infesting faba bean cultivar (Giza 843) produces 1320 seeds/capsules. In one healthy capsule, 4721.49 ripe seeds had been counted and an average of 207, 247 min seeds per plant, 22–49 capsules/plant.

For the weight of ripe seeds, when the letter per column is the same, the data for are not significantly different ($P<0.05$).

Discussion

The occurrence of *Ph. orobanchia* in Egypt seems to be limited. The absence of *Ph. orobanchia* in some fields might be associated with the application of high rate of insecticides. This agrees with the observations of Parker and Riches (1993) and Schroeder (1994) who reported that the use of insecticides against crop pests can largely eliminate *Ph. orobanchia*. The fly population was generally low in the three seasons of the study in February, but increase to its maximum in late March in the 1st season and April in the 2nd and 3rd seasons. The eggs of *Ph. orobanchia* fly are deposited into *O. crenata* shoots and a significant number of fly larvae pupate in the fruit capsules. Up to 19–64 larvae and puparia have been counted in one broomrape ($n=40$ sprouts) in Yugoslavia (Mihajlović 1986). While in this study, in Nubaria, Egypt, the number of larvae and puparia/plant ranged 6–42 fly. In Bulgaria, 100 flowering broomrape sprouts produced 2302 seed capsules (Klein and Kroschel 2002). In the present study, the flowering broomrape shoots ($n=30 \times 3$ reps.) infesting faba bean cultivar (Giza 843) produce 1320 seed capsules per plant. In one capsule, 4721.49 ripe seeds had been counted, and an average of 207, 247 min seeds can be produced per plant. So the broomrapes are very prolific, producing thousands of minute seeds/plant that can be dispersed by field activities and wind. The fly effectively destroyed *O. crenata* capsules. The fly has been found to be efficient in reducing seed capsules of *Orobanch* spp. by 96% in Yugoslavia. The fly has shown effective destruction of *Orobanch* *ramosa* and *Orobanch* *cernua* seed capsules by 81.4% and 71.7%, respectively, under natural conditions at Malima (Ethiopia), (Fessehaie and Parker 1992; Elzein et al. 1999). In Hungary and former USSR reduction in seed production per capsule of *Orobanch* *cumana* on sunflower of 60–85% caused by *Ph. orobanchia* (Sauerborn 1991).

The present findings support these observations. In our study, three larvae per capsule were sufficient to

Table 3 Effect infestation level by *Ph. orobanchia* fly larvae on reducing seed production in *O. crenata* capsules

Insect no./capsule		Capsule weight (mg)	Ripe seeds/capsules		Seed reduction
			Weight (mg)	No. of seeds	
None	Range	19.7–130.1	7.1–50.7	1347.5–9622.1	0.0
	Average (± SE)	63.1 ± 8.1	24.85 ± 3.1 ^a	4721.49 ± 590.5	
1	Range	7.9–54.4	0.0–35.6	0.0–6756.3	59.604
	Average (± SE)	29.93 ± 4.6	8.85 ± 2.6 ^b	1907.3 ± 669.08	
2	Range	11.1–44.3	0.0–6.6	0.0–1252.57	91.36
	Average (± SE)	22.5 ± 3.5	2.15 ± 0.9 ^c	408.03 ± 171.6	
3 or more	Range	7.8–44.1	0.0	0.0	100
	Average (± SE)	28.1 ± 3.7	0.0 ^d	0.0	

completely destroy the contents, while one larva caused partial seed loss. These results align with earlier findings from the USSR and other regions (Okazova 1973; Girling et al. 1979). Hence, the reduction of *O. crenata* seed production by *Phytomyza* fly prevents supplementary infestation and dissemination. Infestation with *Phytomyza* is efficient in reducing *O. crenata* seed yield and may have a great effect in the reduction of *O. crenata* seed bank. In this study, 65.5% of the larvae and pupae were found in stems of *O. crenata*. Significant number of *Ph. orobanchia* pupae remained in dried seed capsules and in stem base of *O. crenata* in Nubaria and Abies farms, could be subjected to predatory arthropods including soil ants (Hegazi et al. 2024b).

Conclusion

The parasitic weed, *Orobanche crenata* is a noxious root parasite seriously damages the production of faba bean *Vicia faba* in Alexandria, Egypt. Significant yield losses in faba bean fields can be attributed to its parasitism. The agromyzid fly *Phytomyza orobanchia* is an effective biological control agent of the weed. Most larvae and puparia of *Ph. orobanchia* fly were found in capsules and plant base of the weed. The fly population was generally low in February but increased to its maximum in late March or April feeding only on *O. crenata* weed. The fly is very efficient in reducing *O. crenata* seed yield and seed bank in infested soil. These findings highlight the potential of *Ph. orobanchia* to be integrated into broader biological control strategies. With the development of suitable field mass-release techniques, this fly could be deployed at scale to sustainably manage *O. crenata* in Egyptian agriculture.

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Author contribution

E. M. H. H. conceived the idea, wrote the first draft of the manuscript and general supervision of the research group, and was a major contributor in writing the manuscript. W. E. K., S. M. A., S. F. A. S., M. A. A., M. A. H., A. M. S., M. A. E. and M. M. A. F. collected the data and participated in its design and coordination and aided to draft the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interests

The authors declare no competing interests.

Ethics approval and consent to participate

Not applicable.

Consent for publication

The manuscript has not been published in completely or in part elsewhere.

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