Research Article

Assessment of Effectiveness among Native Bees in Enhancing *Trifolium alexandrinum* Seed Production

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Abstract | *Trifolium alexandrinum* L. is a multi-cut, winter annual fodder crop in the South Punjab region of Pakistan. Being cross pollinated crop, better seed production of *T. alexandrinum* depends upon insect pollinators. Current study was planned to find effective native insect pollinators which play a crucial role in enhancing seed production of *T. alexandrinum*. The goals were achieved by recording the diversity of insect pollinators, abundance, foraging activity and most importantly pollination efficiency in a single visit in terms of reproductive success parameters (head size, florets per head, seed count/head, seed mass/head and seed mass of 1000 seeds). The pollinator's community was composed of 06 Hymenoptera, 03 Diptera and 02 Lepidoptera species. It was found that the total abundance of a solitary bee *Pesudapis oxybeloides* was higher followed by a honey bee, *Apis mellifera* and a syrphid fly, *Eristalinus aeneus*. Moreover, solitary bee *P. oxybeloides* was detected as the most efficient pollinator based on rate of visitation and pollinator efficiency in a single visit, followed by *A. mellifera* and *A. dorsata*. The seed set in open pollination (free insect visits) was 58 and 70% higher and seed weight of 1000 seeds respectively as compared to self-pollination (no insect visits). Hence, conserving these most efficient native pollinators can lead to higher seed yield of *T. alexandrinum* and other seed crops in South Punjab, Pakistan.

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Introduction

Trifolium alexandrinum L. is a highly important forage crop that is cultivated extensively worldwide, with a particular significance in Pakistan (Arshad *et al*., 2018). In Pakistan, it is grown on almost 78% of total fodder grown area in the Rabi season (Khan *et al*., 2012). *T. alexandrinum* is known as the Fodder king, because, it is also the most popular fodder due to its highly nutritive value, continuous fodder supply from October to April, easy digestibility and role in improving fertility of the soil (Bakheit, 2013). In Pakistan, the total requirement of seed for all fodder crops is 61 thousand metric tons while the availability of seed from all sources (including local production and import) is 24 thousand metric tons so still, there is a deficit of 37 thousand metric tons to meet the local demand (Pakistan Economic Survey, 2020).

Pollination is a keystone process for the reproduction of plants. Almost 85% of plants are dependent on insect pollinators, especially bees, for their reproductive success. About 20,000 bee species have been reported that are providing pollination services worldwide (Ollerton *et al.*, 2011). Solitary bees are one of the most essential pollinators of many agronomic crops, and they also contribute to global food security in a sustainable way (Mallinger and Gratton, 2015; Kleijn *et al.*, 2015). According to estimates, the value of solitary bees' agriculture pollination services is approximately $150 billion (Gallai *et al.*, 2009).

T. alexandrinum is an entomophilous cross-pollinated plant, dependent upon insect pollinators for better seed production (Muhammad *et al.*, 2014). In previous studies, honey bees (*Apis mellifera* and *A. dorrsata*) (Sharma and Singh, 2003; Singh *et al.*, 2012) and solitary bees (*Osmia rufia*, *Megachile rotundata*) proved to be the most effective pollinators of *T. alexandrinum* (Pinzauti and Martiniello, 2003; Mazeed and Zidan, 2019). Moreover, solitary bees have also been reported as the efficient pollinator in other fodder crops i.e., Lucerne (Cane, 2002; Wang, 2009) and Junter (Sajjad *et al.*, 2008). Among the solitary bees, *Pseudapis oxybeloides* (Parker *et al.*, 1986), a soil-nesting bee has been reported as an abundant pollinator from different host plants (*Achyranthes aspera* and *Launaea procumbens*) in the sub-tropical planted forest of Southern Punjab (Sajjad *et al.*, 2019) and also from lufa guard flowers (Ali *et al.*, 2016).

The current study was planned to find out effective native insect pollinators that enhance seed yield in *T. alexandrinum*. Moreover, no single study has previously revealed the pollinator efficiency in a single visit in the production of *T. alexandrinum* seeds in South Punjab, Pakistan.

Materials and Methods

Site of experiment

The study was carried out in MNS University of Agriculture, Multan, Pakistan (30.1475° N, 71.4436° E) during the vegetative season November to May in 2019-20. The selected cross-pollinated crop, *T. alexandrinum* was grown on a 0.125 acres area.

Diversity and abundance of native insect pollinators

The abundance and visitation frequency (number of visits per flower per minute) of native insect pollinators were evaluated for the whole of flowering period of the *T. alexandrinum* crop (from 2nd week of April to 1st week of May) after every two days. On each observation day, data was recorded at two time periods i.e., 0800 hr and 1600 hr. During each time period, arbitrarily 25 plants were selected and each plant was individually observed for the time period 60 sec to record all the native insect pollinators visiting the *T. alexandrinum* flower. Some of the insect pollinators were also caught for later taxonomic identification by using taxonomic keys (Vockeroth, 1969; Michener, 2000).

Pollinator's foraging behavior

In order to compare effective pollinators in *T. alexandrinum* foraging behavior in terms of visit duration (time spend by individual pollinator/flower/visit) and visitation rate (no. of flowers visited by a single pollinator/min) were recorded for most abundant insect pollinators. Observations were recorded after every three-day interval for the whole peak flowering period (20th April to 15th of March) at two time period (0800 hr and 1600 hr) (Sajjad *et al.*, 2008).

Pollinator effectiveness

To determine the effectiveness of pollinators in terms of quantity of seeds produce during a single visit of pollinator, the buds of the experimental plant which were not opened were caged with fine mesh. After opening of buds, the mesh bag was removed at peak pollinator abundance time. Afterwards, after a single visit of a pollinator the buds were caged once again until the senescence of the flower occurred. Different parameters of the reproductive success were recorded after the harvesting of pods in terms of head size (cm), seed count/head, seed mass/head (g), florets/head and seed mass of 1000 seeds (g).

Furthermore, around 30 plants were arbitrarily selected for each open pollination treatments (free insect visitation) and caged treatments (no insect visitation) were also kept for comparison of reproductive success parameters.

Data analysis

To compare the effectiveness of pollinator in terms of visit duration, visitation rate and seed set efficiency in a

Table 1: *Insect pollinator species visiting berseem flowers with their total abundance and visitation frequency.*

Order	Family	Genus/species	Total abundance	Visitation frequency (individuals/plant/min)
Hymenoptera	Halictidae	*Pseudapis oxybeloides*	291	0.38
	Apidae	*Apis mellifera*	203	0.27
		Apis dorsata	98	0.13
		Apis florea	79	0.11
		Xylocopa sp.	49	0.07
	Vespidae	*Vespa orientalis*	30	0.04
Diptera	Syrphidae	*Eristalinus aeneus*	167	0.23
		Episyrphus balteatus	67	0.09
		Ischiodon scutellaris	25	0.032
Lepidoptera	Nymphalidae	*Vanessa cardui*	21	0.03
	Erebidae	*Utetheisa pulchella*	27	0.036

single visit, analysis of variance (ANOVA) was used to determine the parameters of reproductive success. All the means were compared by LSD test at P = 0.05. All the statistical analysis was done in Statistix 8.1 (Statistix, 2005).

Results and Discussion

In the total duration of peak *T. alexandrinum* flowering, pollinator community was consisted of five bee species, three syrphid fly species, and a single species each of wasp, butterfly and moth. The majority of the total abundance observed in the results was made up of bees (Hymenoptera) accounting for 70% and syrphid flies (Diptera) accounting for 25% (Figure 1). Moreover, the solitary bee, *P. oxybeloides* (Halictidae) was the most abundant insect pollinator followed by honey bee *A. mellifera*. Average visitation frequency was also found to be the highest for *P. oxybeloides* followed by *A. mellifera* and *E. aeneus* (Table 1). However, other pollinators i.e., butterfly, moth and wasp species were recorded least abundant in experimental crop (Table 1).

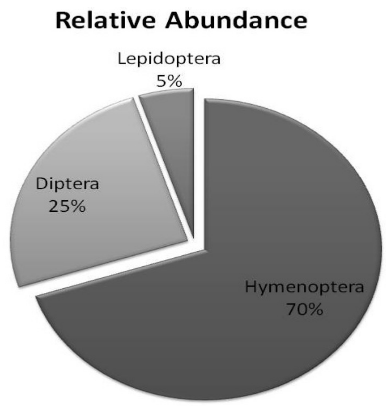

Figure 1: *Relative abundance of insect pollinators visiting berseem flowers.*

The visit duration (F=2.69, df=3, P=0.047) revealed that *A. florea* spent the most time per head (19.21±7.36 sec) followed by *E. aeneus* (5.38±0.39 sec) and *A. dorsata* (12.11±1.23 sec). However, visitation rate (F=45.0, df=3, P<0.0001) was highest for *P. oxybeloides* (17.27±0.67) followed by *A. mellifera* (11.33±1.32) while it was lowest for *E. aeneus* (4.6±0.35) and *A. florea* (5.12±0.67) (Table 2).

Table 2: *Pollination effectiveness of insect pollinators in terms of visitation rates and visit duration.*

Pollinator species	Visitation rate no. of flower visit/min (N=50)	Visit Duration / flower/visit (N=50)
P. oxybeloides	17.27 ± 0.67 a	11.03 ± 3.07 bc
A. mellifera	11.33 ± 1.32 b	10.68 ± 0.99 c
A. dorsata	6.57 ± 0.27 c	12.11 ± 1.23 b
A. florea	5.12 ± 0.67 d	19.21 ± 7.36 a
Xylocopa sp.	7.27 ± 0.88 c	5.32 ± 1.25 d
E. aeneus	4.6 ± 0.35 d	13.38 ± 0.39 b

Mean values sharing similar letters in respective columns show non-significant differences according to LSD at the 5% level (±SE).

There was significant difference among the insect pollinators based on single visit reproductive success parameters in *T. alexandrinum* i.e., head size (F=77.0, df=3.0, P<0.0001), no. of seed/head (F=24.4, df=3.0, P<0.0001), seed weight/head (g) (F=33.4, df=3.0, P<0.0001), floret/head (F=24.8, df=3.0, P<0.0001), and 1000 seed weight (g) (F=33.7, df=3.0, P<0.0001). The results shows that solitary bee *P. oxybeloides* was the most efficient pollinator based on seed set/ single visit followed by honey bees *A. mellifera* and *A. dorsata*. Moreover, open pollination (free insect visits) resulted in higher seed mass/head (85%), seed count/ head (58%) and 1000 seed mass (70%) as compared to caged treatment (no insect visits) (Table 3).

Table 3: *Seed setting resulting from single visits of abundant pollinators vs open and self pollinated.*

Pollinator species	Head size (cm)	Floret/ head	No. of seed/ head	Seed weight/ Head (gm)	1000 seed weight
P. oxybeloides	1.84 ± 0.07 b	49.55 ±2.35 b	49.22 ± 2.93 a	0.29 ± 0.01 a	3.77 ± 0.19 a
A. mellifera	1.33 ± 0.09 bc	42.22 ± 2.64 c	26.67 ± 1.35 b	0.28 ± 0.02 b	3.0 ± 0.20 b
A. dorsata	1.43 ± 0.03 bc	31.22 ± 2.64 c	30.67 ± 1.55 b	0.20 ± 0.02 b	2.69 ± 0.29 bc
E. aeneus	1.13 ± 0.09 c	28.22 ± 3.14 d	26.22 ± 1.51 b	0.12 ± 0.01 c	1.79 ± 0.20 c
Open pollinated	2.42 ± 0.05 a	58.51 ± 2.88 a	57.20 ± 2.86 a	0.34 ± 0.02 a	4.20 ± 0.22 a
Self pollinated	1.03 ± 0.05 c	21.06 ± 2.15 d	23.53 ± 1.13 c	0.05 ± 0.01 d	1.22 ± 0.11 c

Mean values sharing similar letters in respective columns show non-significant differences according to LSD at the 5% level (±SE).

In this study, bees (69%) were the more abundant pollinators compared to flies and other insect pollinators (31%). Among the bees, *P. oxybeloides* was the most abundant and *A. mellifera* and *A. dorsata* followed respectively. Moreover, *E. aeneus* was also among the top three abundant insect pollinators. A previous study also revealed that higher abundance was found for solitary bees (*Megachile* sp.) in *T. alexandrinum* (Dimitrov *et al*., 2020) while most of the other studies have reported honey bees (*A. dorsata* and *A. mellifera*) as most abundant pollinators visiting *T. alexandrinum* flowers (Sharma and Singh, 2003; Singh *et al*., 2012; Jat *et al*., 2014; Latif *et al*., 2014). Moreover, previously some studies reported that solitary bees are more abundant in fodder crops i.e., lucerne and Jantar (Cane, 2002; Sajjad *et al*., 2009; Wang *et al*., 2009).

In this study the visitation rate of solitary bee *P. oxybeloides* was higher followed by honey bees *A. mellifera* and *A. dorsata*. Contrarily, some studies have reported higher visitation for honey bees (*A. florea, A. dorsata*) in *T. alexandrinum* (Shivrana, 1996; Jat *et al*., 2017). However, visitation rate was found higher for a solitary bee (*Megachile* sp.) in other fodder crop i.e., lucerne (Cresswell, 2008). There was no statistical difference were observed in visitation rate of honey bees and solitary bees in Jantar crop (Sajjad *et al*., 2009).

Furthermore, the seed set by single visit is highly important parameter to assess the efficiency of insect pollinators as compared to pollen harvest per single visit (Ali *et al*., 2016). Our study showed that *P. oxybeloides* is the most effective insect pollinator in term of single visit seed set followed by *A. dorsata* and *A. mellifera*. Previously in beseem, no study has shown the seed set efficiency in a single visit. However, some other studies have shown the relative effectiveness (in terms of seed set) of solitary bees and bumble bees over honey bees under caged plot treatments (Pinzauti and Martiniello, 2003; Cecen *et al*., 2007; Mazeed and Zidan, 2019). Some other studies have also reported that solitary bees are most efficient in seed production of other fodder crops i.e., Jantar (Sajjad *et al*., 2009) and lucerne (Cresswell, 2008). Moreover, in open pollination (free insect visits), seed mass per head, seed count/head and seed mass of 1000 seeds were higher than the caged treatment (no insect visits). *T. alexandrinum* is largely insect pollinator dependent crop and bees have been found to increase seed set up to 50-73% as compared to no insect visit treatment (Bharadwaj and Kumar, 2005; Singh *et al*., 2012; Bondok *et al*., 2016).

Conclusions and Recommendations

In conclusion, conserving native solitary bees can enhance *T. alexandrinum* seed production and can also contribute positively to the yields of other cross-pollinated crops. Provisioning of year-round floral resources can help to enhance the diversity of wild insect pollinators (Nicholls and Altieri, 2013) leading to higher seed/fruit yield.

Novelty Statement

To best of our knowledge, no prior research work has been investigated efficiency of Solitary bees in single visit in *T. alexdanrinum*, highlighting a critical knowledge gap in our understanding of their reproductive success and comparison of Solitary vs social bee.

Author's Contribution

Mudssar Ali, Asif Sajjad and Muhammad Awais Ahmad: Conceived and designed the experiment.
Muhammad Awais Ahmad and Mudssar Ali: Conducted the experiment.

Muhammad Awais Ahmad, Mudssar Ali and Shafqat Saeed: Provided the analysis tools and analyzed the data.
Muhammad Awais Ahmad and Mudssar Ali: Wrote the research article.
All authors contributed to manuscript review and editing.

Conflicts of interest
The authors have declared no conflict of interests.

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