

**“To study the pollination behaviour and effect of  
honey bee pollination on quantitative and  
qualitative parameters of Egyptian Clover  
*Trifolium alexandrinum* L.”**



**THESIS**

*submitted to the*

**Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya**

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**MASTER OF SCIENCE**

*In*

**AGRICULTURE**

**ENTOMOLOGY**

*by*

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**2021**

## CERTIFICATE - I

*This is to certify that the thesis entitled, “To study the pollination behaviour and effect of honey bee pollination on quantitative and qualitative parameters of Egyptian Clover *Trifolium alexandrinum* L.” submitted in partial fulfillment of the requirements for the Degree of **MASTER OF SCIENCE in Agriculture Entomology of Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior**, is a record of the bona-fide research work carried out by **Mr. KANHAIYA LAL KUMAWAT** under my guidance and supervision. The subject of the thesis has been approved by the student’s Advisory Committee and the Director of Instruction.*

*No part of the thesis has been submitted for any other degree or diploma or has been published. All the assistance and help received during the course of this investigations has been acknowledged by the scholar.*

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*This is to certify that thesis the entitled “To study the pollination behavior and effect of honey bee pollination on quantitative and qualitative parameters of Egyptian Clover *Trifolium alexandrinum* L.” submitted by Mr. KANHAIYA LAL KUMAWAT to the Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE in AGRICULTURE** in the department of **Entomology**, College of Agriculture, Gwalior has been accepted after evaluation by the External Examiner and approved by the Student’s Advisory Committee after an oral examination of the same.*

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Place : Gwalior

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## List of Abbreviations and Symbols

Symbol	Abbreviation	Stand for
@		At the rate of
%		Per cent
±		Plus or minus
°C		Degree centigrade
	CV	Coefficient of variation
	CD	Critical difference
	d.f.	Degrees of freedom
	<i>et al.</i>	Co- workers
	Fig.	Figure
	RH	Relative Humidity
	NS	Non-significant
	spp.	species
	viz.	For instance
	i.e.	That is
	etc.	X sectra
	kg.	kilogram
	gm	gram
	cm	centimeter
	mg	Milligram
	hrs.	hours
	q	quintal
	r	Correlation coefficient
	<i>viz.,</i>	Namely

## Chapter I

# INTRODUCTION

Egyptian clover (Berseem) is one of the important winter annual legume known as king of fodder crop, is popular among all the classes of livestock farmers of the world. It belongs to the clover group and internationally famous as Egyptian clover. It has spread very fast after its introduction in 1903. Botanically it is known as *Trifolium alexandrinum* L. Berseem is one of the oldest cultivated clovers, domesticated in Egypt and later introduced into many other parts of the world. It is adaptable to a wide range of soil and environmental conditions and combines well with many perennial grasses.

Being the principle source of forage, and for conserving soil fertility under intensive cropping, it is considered a good source of protein, phosphorus, calcium, and other minerals for animals and a source of nectar and pollen for bees so that it has played a key role in the development of meat, milk, poultry, beekeeping and related industries.

Livestock animals prefers this crop over other as a forage as it holds higher value quantitative characteristics such as green fodder yield (40 t/fed) and in multicut nature (4-7 cuts) along with higher qualitative characteristics such as succulency, high palatability and nutritive value, digestibility up to 65% and continuous supply of 7 months (Oct to May). On dry matter basis, it contains 17-22 % crude protein, 42-49 % neutral detergent fibre, 35-38 % acid detergent fibre, 24-25 % cellulose and 7-10 % hemi cellulose. The green forage can also be converted in to excellent hay and utilized for enrichment of poor quality roughages from cereals (IGFRI Jhansi, 2012). Ten to fifteen kg of fodder alone with straw constitutes a maintenance ration. It can support growth and milk production on *ad lib* feeding, balanced by straws.

Among the berseem growing countries, India is having highest area under berseem with around 2 million ha followed by Egypt (1.1million ha) and Pakistan (0.71 million ha) (Muhammad *et al.*, 2014). Berseem is one of the major *Rabi* forage crops in northern region of India and occupies maximum area among forage crops during winter season (Hazra, 1995). In North India, mainly in four states the berseem production is in large tracts. The states of Uttar

Pradesh, Madhya Pradesh, Punjab and Haryana produce berseem to the maximum extent.

Utilization of pollinators, especially honey bees is considered as one of the cheapest and ecofriendly approach in maximizing the yield of cross pollinated crops (Free, 1993). Many experiments have consistently confirmed that the yield levels can be increased to the extent of 50 to 60 per cent in fruits and plantation crops, 45 to 60 per cent in berseem, sunflower, sesamum and Niger and 100 to 150 per cent in cucurbitaceous crops. With this, beekeeping has achieved an important economic status and contributes significantly to national income. Traditional beekeeping methods are also continue to be common in tribal parts and many other parts of the country. Honeybees are well known for pollinating a wide range of crops and assisting in the conservation and preservation of biological diversity. With this they provides a variety of useful products such as pollen, honey, royal jelly, bee wax, propolis, and bee venom. (Johannes Meier and Mostert, 2001)

Approximately 3/4<sup>th</sup> of the world's flowering plants subject to pollination by insects (Bezabih and Gebretsadikan, 2014). Egyptian clover is one of the most entomophilic crop requiring insects, especially bees for cross – pollination. The western honey bee, *Apis mellifera* L., is the most common and popular pollinator in India.

Forage crops are absolutely dependent on insect pollination. *T. alexandrinum*, a predominantly cross- pollinated legume is an important fodder crop. For better seed setting, tripping of flowers by any pollinating agent is needed in *T. alexandrinum*. Effectively planned and well managed bee pollination ascertains the crop's quality and quantity. Pollination is closely associated with fruit and seed set, yield and seed quality. Nowadays intensive agriculture plays major significant role in declining native local pollinators through the alteration, elimination and modification of pollinator habitats and indiscriminate application of agricultural chemicals (fertilizers, pesticides and herbicides) Kearns *et al.*, 1998. Intensive cultivation of land will destroy many natural food sources and nesting sites of wild pollinating insects Free (1993).

*T. alexandrinum* is also an important honey producing crop and provides a good source of pollen for bees. A flower head consists of 50 to 250 (average of 100) florets. Each floret most commonly contains 5 or 7 ovules, but under

normal conditions only about 50% of the ovules develop into seeds (Pasumarty *et al.*, 1993). Besides the genotype of white clover, various environmental and seasonal factors are involved in attaining maximum yield (Marshall, 1994). Inadequate pollination is a frequently cited as the cause for poor seed yields (Fairey, 1993). The most important pollinator of *Trifolium* spp. are bees. There are varied benefits derived from bee pollination in *Trifolium* spp. such as increased seed production. (Williams and Carreck, 1994). Honey bees (*Apis mellifera* L.) are the most effective pollinators for seed set of clovers and they comprise 88% of all insect visitors to this crop (Goodman and Williams, 1994). Management of honey bees is easier than others, and as a result they are the pollinator of choice in the major clover seed production areas of the world.

Entomophily is also a key factor in the sexual reproduction of many agricultural crops where the importance of pollinators is becoming more recognized for enhancing pollination efficiency and therefore yields and quality of major seed and food. It has been estimated that about 30 per cent of the human food is derived from bee pollinated crops (Slaa *et al.*, 2006). Pollination often becomes a constraint in insect pollinated species and results in a marked reduction in crop productivity. The reasons for inadequate pollination includes reduction in the population of native pollinators because of loss of habitat of insects, extensive use of pesticides and herbicides and a considerable increase in the level of pollutants for which insect are very susceptible.

Honey bees foraging for the search of nectar and pollen often cause tripping of the berseem flowers and thus facilitate the process of pollination. Nectar is the basic carbohydrate or energy source, and pollen is the basic protein source for the pollinator insects. Pollination regulates a suite of developmental responses that transform the flower from a structure dedicated to pollen dispersal and reception to one that is dedicated to fertilization, embryogenesis, seed development, and ultimately seed dispersal.

Foraging is an important activity carried out by honeybees which is responsible for collection of nectar and transfer of pollen grains. This can result into higher efficiency of pollination directly leading to the higher crop yields. Attempts were, therefore, made to regulate the visits of honeybees so that they can be made to visit only the plants of interest. Use of honeybee attractants is very important from this point of view. Honeybees mainly communicate with

each other by means of vision, taste, touch and smell. Success of pollination with the help of honey bees depends on their performance on the target crop over other competing plants in the vicinity. Many substances have been tested as honey bee attractants.

Bee attractant is any substance or material that encourages honey bees to visit certain crops. Sugar solution, Coriander extracts, Jaggery solution, Cacambe, Onion Solution, Sugarcane juice, Bee-Q, etc. can be used diluted in water as honeybee attractants.

El-Shahawy and Gheit, 2001 and Abdalla *et al.*, 2008 Studies on Egyptian Clover (berseem) reported that pollination and seed setting was highest under open field and caged with bee hive than caging without bee and selfing (without tripping) conditions. The introduction of honey bee to berseem grown plots improved the seed setting, which indicated that berseem is self-compatible but needs stimulating agents (honeybee or tripping) to set seeds due to relative position of male and female organs and the presence of bubbles on stigma.

Singh *et al.*, (2012) also reported higher seed yield per plot (18 m<sup>2</sup>) in plots caged with *Apis mellifera* colony (2-frame), (4-frame) and (6- frame) in comparison of caged without bees or without insect pollination (WIP). Honey bees are found to be the prime and most essential pollinating agent of berseem visiting it avidly for pollen and significantly increasing seed yield according to Sharma and Singh, (2003) *Apis dorsata* (6.5 bees) was the most efficient flower visitor followed by *Apis mellifera* (4.4 bees). Having such a large potential for increasing Egyptian clover biomass and seed output, man used a number of pollinators for guided pollination, including *A. mellifera* and *A. cerana* (Pinzauti and Martiniello, 2003).

So, going through all the facts and information discussed above, this study was aimed to determine the pollination behaviour of *Apis mellifera* L. and other major pollinators visiting berseem crop during the flowering season and also to make the comparison of effect of pollination on the qualitative and quantitative parameters of seed production and hence the study was made with the following objectives:-

**Objectives:-**

1. To study the pollinators fauna of Egyptian clover.
2. To study the effect of bee attractant to attract the bees in Egyptian clover.
3. To study the Effect of bee pollination on quality and quantity parameters of Egyptian clover.

## Chapter II

### REVIEWS OF LITERATURE

A successful crop production greatly depends on the efficient pollination in the flowers. This results in the higher economic and biological yield. An efficient pollination in different crops, vegetables and fruits by different pollinators plays role in increasing nutrition and aids in fruit setting. Several studies have been conducted earlier related to all these aspects of role of pollination in crops due to insects, i.e. entomophily. The available literature on the pollinators' fauna, effect of bee attractants to attract the bees and the effect of bee pollination on quality and quantity of Egyptian clover is reviewed in this chapter under the following heads:

#### **Egyptian Clover and its pollinators' fauna.**

#### **Effect of bee attractants to attract the bees in Egyptian clover.**

#### **Effect of bee pollination on quality and quantity parameters of Egyptian clover.**

#### **Egyptian Clover and its pollinators.**

Weaver (1957) and Rodet *et al.*, (1998) reported that one strong honey bee colony can visit all blossoms in a 1 ha clover field resulting in 70% fertilization under ideal conditions.

The major predators and pollinators of Egyptian clover include the Coccinellidae, a syrphid fly and honey bees. (Shamel, 1956; Naryann *et al.*, 1961; Hussnain 1953).

Dixit *et al.* (1989) reported that berseem clover flowers are largely self-sterile and insects play an important role as pollinators in cross-pollination. Up to 82% of Berseem clover plants can be cross-pollinated by honeybees.

Omran (1992) reported that in Qena region (Upper Egypt) *Apis mellifera*, *Megachile submucidae* and *Andren fuscosa* the most abundant hymenopterous visiting the Egyptian clover flowers.

Williams and Carreck (1994) reported that the most important pollinator of *Trifolium* spp. are bees. There are varied benefits derived from bee pollination in *Trifolium* spp. such as increased seed production.

Chaudhary (1998) who reported that insect pollination in crops is the most critical ecosystem service fulfilling plant's ultimate requirement of pollination, fertilization, seed setting and seed production.

Sihag *et al.* (1999) studied the foraging pattern of *A. dorsata*, *A. mellifera* and *A. florea* on eight cultivars of oil seed crop and reported that in oil seeds, the honey bee visitation frequency was low in the morning and reached peak between 1100 to 1300 hr and again declined in the evening.

In several regions, the honeybee seems to be the main insect pollinator of many cultivated crops (Michener, 2000; Tchuenguem *et al.*, 2000). Bees pollinate one-sixth of the world's flowering plants while one-third of the food consumed is pollinated by honeybees (MAAREC, 2000).

Chandel *et al.* (2000) reported that *A. cerana* was observed to start its foraging activity at 0625 hr and *A. mellifera* at 0720 hr on toria. *A. cerana* ceased its foraging at 1730 hr and *A. mellifera* at 1740 hr. Thus, the average duration of foraging was 1118 hr and 1012 hr in *A. cerana* and *A. mellifera*, respectively. The highest number of incoming foragers of *A. mellifera* (90.7) and *A. cerana* (274.3) was recorded at 1000 to 1030 hr at a temperature of 25°C.

Sharma and Singh (2001) noticed the foraging time of *A. florea* and *A. dorsata* in carrot ecosystem. *A. florea* spent more time (37.09 sec/flower) and visited less number of flowers (2.20/min) as compared to *A. dorsata* that visited more number of flowers (4.31/min) and spent less time (9.20 sec/flower).

Shivaramu and Chithiraichelvan (2002) recorded *A. florea*, *Trigona* sp., *A. cerana*, *A. mellifera* and wasp on radish flower with peak activity between 1300 hr to 1600 hr at chettalli.

Sharma and Singh (2003) reported that abundance of *A. dorsata* was maximum (6.55 bees/m<sup>2</sup>/5 min) followed by *A. mellifera* (4.4 bees) and *A. florea* (1.52 bees). *A. dorsata* and *A. mellifera*, however, carried equal number of pollen grains (av. 8125) on their body and *A. florea* entrapped lesser pollen grains (av. 4625). The pollination index of *A. dorsata* was found to be the highest (53219), while it was lowest (7030) in case of *A. florea*. Hence, it

delineates that *A. dorsata* was most efficient pollinator of Egyptian clover followed by *A. mellifera* and *A. florea* under agro ecological condition of Hisar.

Pandey and Tripathi (2003) reported that *A. cerana indica* showed the highest frequency of visits followed by *A. dorsata*, *A. mellifera* and *A. florea* in mustard. Regarding the time spent of *A. dorsata* worker took the least time followed by *A. mellifera*, *A. cerana indica* and *A. florea*.

Pinzauti and Martiniello (2003) reported the immense scope to increase production of biomass and seed of Egyptian clover, a variety of pollinators has been used by the man for guided pollination that included *A. mellifera* and *A. cerana*.

*Apis dorsata* and *Apis florea* tend to migrate locally in response to the availability of floral resources and have the ability to increase rapidly in number in response to flowering events (Itioka *et al.*, 2001). Honey bees require carbohydrates, proteins, fats, minerals, vitamins, and water for the maintenance, growth and development of their colonies (Loper & Berdel, 1980). These elements are sourced by flowering plants in shape of pollen and nectar. However, the foraging activity of honey bees is greatly influenced by the weather conditions and availability of nectar and floral resources (Neupane & Thapa, 2005).

In an open field cultivation system, insect pollinators are mainly attracted to flowers of plants or crops for resources such as pollen, resin and nectar or extra nectaries which they use as sources of energy and food as well as, constructing their nests (Karikari and Kwapong, 2007).

Klein *et al.* (2007) who reported that 87 of the 115 leading global food crops depend upon animal pollination and forage crops are absolutely dependent on insect pollination.

Murray *et al.* (2009) and Hepburn and Radloff, (2011) In Pakistan, due to the widespread use of neonicotinoid pesticides, such as imidacloprid and thiamethoxam, honeybee populations are declining rapidly and this would be having significant impact on the pollination and subsequent yields of Berseem clover seed.

Pollination improves seed quality by increasing seed weight; lack of pollination has consistently been identified as a major limiting factor to higher and more reliable seed yields (Saeed *et al.*, 2011).

Hesain (2013) recorded eleven species of bees and other insects on the flower of the clover, which are four species from hymenoptera, (*Apis mellifera*, *Megachil sp.*, *Halictus sp.* and *Anderena sp.*), three species of diptera were (*Metasyrphus taeniops*, *Eristalis anaeus* and *Episyphus balteatus*) and four species of Lepidoptera it were (*Lycaeides Melissa* (alpime), *Autograph gamma* L., *Colias croceus* L., and *Artogeia rapa*).

Hameed *et al.* (2016) reported that armyworm, *Spodoptera exigua*, aphid (*Sitobian avenae* L.) and leaf miner (*Aproaerema modicella*) are important pests of berseem while the *Coccinellids* and a *Syrphid* fly are important predators and honey bee are important pollinator of berseem.

Mabrouk and Mahbob (2017) recorded in the Egyptian clover field twenty two insect species, including five species of pests, five species of natural enemies and twelve of pollinators (*Phillian thusabdelkader*, *Polistesgallica*, *Vespa orientalis*, *Megachi leuniformis*, *Osmia spp.*, *Chalcido masiculum*, *Apis mellifera* L. *Andrena spp.* and *Ocnera hispida*)

Sajjad *et al.* (2017) reported that the most visited plants species by *A. dorsata* included *Helianthus annuus*, *Citrus reticulata*, *Trifolium alexandrinum*, *Moringa oleifera* and *Calotropis procera*, while the most visited plant species by *A. florea* included *C. procera*, *Mangifera indica*, *T. alexandrinum*, *Coriandrum sativum* and *H. annuus*.

Singh *et al.* (2005) and Mohapatra *et al.*, (2010) reported that being the pollination service provider bees contribute handsomely in enhancing the productivity and production of cross pollinated crops through efficient pollination in an inconspicuous and silent manner.

Hepburn and Radloff (2011) reported that there are different kinds of honeybees found in Pakistan including *A. florea*, *A. cerana*, *A. dorsata* and *A. mellifera*, however, *A. mellifera* in particular contributes significantly in the pollination process (Taha and Bayoumi, 2009 and Meena *et al.*, 2015).

There are different factors which influence the activity of honeybees in crop pollination. Distance from the hive, high temperature and humidity all reduce honeybee activity and thus indirectly reduce seed yields. In a field study conducted at Haryana in India, Jat *et al.*, (2014a) found that *Apis dorsata* was the most abundant floral visitor on Berseem clover followed by *A. mellifera*. Honeybee visitation during summer was greatly influenced by time of the day and wind speed; with maximum visits in the afternoon (14:00, 12:00 and 16:00 h) and minimum visits during early morning (at 06:00 and 08:00 h). The activity of both *A. dorsata* and *A. mellifera* was also influenced by relative humidity (RH) and was positively correlated with temperature and time of the day.

*Apis mellifera* was imported to the agricultural lands of Punjab province in the 1960s and has become very popular among beekeepers. It is an effective pollinator of Berseem clover flowers (Meena *et al.*, 2015).

#### **Effect of bee attractants to attract the bees in Egyptian clover.**

Patil *et al.* (2000) reported that the study of spraying of Bee-Q and Bee here on sesame shows that increase in bee visitation and yield parameters significantly on sprayed crop up to 5th day in Dharwad.

Kalmath and Sattigi (2002) reported that spraying of Cacambe (10 %) and jaggery (10%) attracted maximum number of *A. dorsata* up to 15 days after first and second spray Bee-Q (1.25%) and sugar solution were the next best attractants.

Viraktamath and Patil (2002) reported that application of Fruit boost and Bee-Q attracted more bees to sunflower than unsprayed crop.

Alshaff (2002) reported that a wide range of pollinators including *A. mellifera* visited umbles sprayed with rose water and spent more time for foraging than they did in unsprayed umbels.

Viraktamath *et al.*, (2002) reported that the research on bee attractants on *Cucumis sativa* proved that 2 applications of Bee-Q (12.5 gm/lit), Bee-here (4 ml/lit) and sugar solution (10 %) on staminate flowers of *Cucumis sativa* enticed a greater number of bees (4.01 to 4.97 bees/flower in 5 min.) up to 5 days after first and second sprays compared to unsprayed crop (3.25 to 3.59

bees). Similarly, higher visitations were recorded on pistillate flowers on the sprayed crop.

Viraktamath and Anagoudar (2002) reported that two applications of Bee-Q® (12.5g/l), Bee-here® (4 ml/l) and sugar solution (10 %) on staminate flowers of *Cucumis sativa* L. noticed more number of bees (4.01 to 4.97 bees/flower/5 min) up to 5 days after first and second sprays compared to unsprayed crop (3.25 to 3.59 bees). Similarly, higher visitation was recorded on pistillate flowers on the sprayed crop.

Naik *et al.* (2003) reported that sugar syrup containing extract of dried fruits of *Fagara budrunga* plant were more attractive to *A. cerana* than sugar syrup. According to Manjunath (2003) spraying of fruit boost and Bee-Q significantly enhanced visitation by *A. dorsata*, *A. cerana*, *A. mellifera* and other pollinators. However, attractants lost their efficacy after 5 days of spraying in sunflower.

Malerbo – Souza *et al.* (2004) reported that Bee – here, eugenol, geranol, citral, and lemon grass extracts diluted in water were effective in attracting bees to sweet orange orchards.

Nidagundi (2004) reported that comparison between different bee attractants and open pollination observed that spraying of cacambe 10 percent, Bee-Q 1.25 percent and jaggery solution 10 percent have significant influence in attracting a greater number of pollinators over open pollination.

Shimaa Essa (2010) observed that bee visitation usually began on clover in the beginning of May till the beginning of June in the two studied seasons. The maximum activity was observed on May 19th (8.03 bees/m<sup>2</sup>/min.), and reached its lowest activity on June 3<sup>rd</sup> (2.26 bees/m<sup>2</sup>/min.) in the 2006 1<sup>st</sup>, while it was 7.79 bees/m<sup>2</sup>/min on May 17th, the minimum was 0.40 bees /m<sup>2</sup>/min) on May 5th in the 2<sup>nd</sup> season.

Jayaramappa *et al.* (2011) reported that the studies on Bee-Q @ 10, 12.5 and 15 gm/lit, Fruit boost @ 0.50, 0.75 and 1 ml/lit, Cinnamon leaf extract @ 5%, Tuberose floral scented water, 10% sugar solution on ridge gourd and crop deprived is control, which is open pollinated observed that spraying of Fruit boost @ 0.5 ml/lit and Bee-Q @ 12.5 gm/lit enhanced foraging activities of rock

bees and yield parameters like number of fruits per plant to 19.00 and 17.00 fruits, when compared to 10.66 fruits per plant in open pollinated plot. Number of fruits was 21.83 and 20.83 fruits per plot, when compared to 15.68 fruits per plot in open pollinated plots.

Venkataramgowda *et al.* (2013) studied on use of bee attractants, Bee-Q and Fruit Boost in the pollination of Niger. Bee visitations to Niger flowers were observed for two weeks and an estimation of seed yield was determined. Results indicate that applications of Bee-Q at 12.5 gm/lit and Fruit boost at 0.75 ml/lit on Niger plots significantly increased the number of bee foragers over control plots. In addition, plots sprayed with these bee attractants significantly enhanced the seed set, seed weight, and germination of Niger.

Naik Anjan Kumar *et al.* (2014) studied bee attractants play a beneficial role in enhancing pollination and yield of crops especially when target crop is not so attractive to the bees naturally or when the weather conditions are not conducive for foraging by the bees on target crop and evaluated that Citral E, Citral Z, *F. budrunga*, *S. densifolia* attracted significantly a greater number of bees with 2.13 to 2.96 bees /10m<sup>2</sup>/5 min. Which were on par with each other and were as good as Fruit boost showed 2.00 to 2.17 bees /10m<sup>2</sup>/5 min.

Jayaramappa *et al.* (2015) reported that the usage of bee attractants, Bee-Q and Fruit Boost in the pollination of watermelon at different concentrations and indicated showed that, Bee-Q at 12.5 gm/lit and Fruit boost at 0.5 ml/lit of watermelon plots meagrely attracted several bee foragers than the control plots.

Kumari and Rana (2018) reported that the results revealed that one day before both sprays of bee attractants (first at 5% flowering and second after 10 days of first spray), the number of insect pollinators of each group (bees, syrphids and other pollinators) visiting the onion bloom did not differ significantly among the treatments. A after both sprays, Bee Scent @ 7.5% proved best in attracting higher number of insect pollinators of each group up to third day followed by Bee Scent @ 5%, Bee Scent @ 2.5%, followed by sugar solution @ 10% and honey solution @ 2%. The least number of insect pollinators of each group were recorded in open pollination without spray (untreated control).

Manchare *et al.* (2020) reported that the bee attractants were sprayed two times, first at 10 percent flowering and second at 50 percent flowering. The results from the foraging activity of bees noted that the intensity of *Apis dorsata* was increased on 1 day after spraying and it reduced as gone towards 7 days after spray. Spraying of bee attractants i.e. honey solution 10 percent, jaggery solution 10 percent and molasses 10 percent attracted the maximum number of *Apis dorsata* up to 5th day after first spray and 7<sup>th</sup> day after second spray.

### **2.3 Effect of bee pollination on quality and quantity parameters of Egyptian clover.**

Hassanein (1953) recorded highest seed setting (38.9%) in BP followed by OP (23.5%) and minimum in WIP (1.9 seeds/head).

Similarly, Narayanan *et al.*, (1961) reported higher (1958 and 2671 seeds/100 flower heads) yield in two plots compared to only 27 to 28 seeds with automatic self-pollination in cages during 1957 and also during 1960 at 4360 and 64 to 43 with WIP.

Chowdhury *et al.* (1966) who reported significantly higher seed setting in plots where hand tripping and open pollination was employed (42.13 and 44.07%) compared to selfing (8.50%).

Verma and Joshi (1983) reported that on mustard bloom, honeybee pollination increased the number of seeds by 4.07 per pod.

Beri *et al.* (1985) who recorded higher seed setting under open pollination (53.0%) compared with WIP (26.3%) and pollinators + hand tripping (52%).

Delbrassinne and Rasmont (1988) reported that intensive pollination of *Brassica juncea* Linn by *A. mellifera* increased the number of seeds per pod (12.22%),

Panda *et al.* (1989) obtained average seed number / pod was 10.80 whereas without insects were 5.90.

Bakheit (1989) found that cross pollination resulted in a 51% increase in seed production of Berseem clover over caged plots.

Prasad *et al.* (1989) they reported in *B. juncea*, open pollination gave the maximum yield (13.4 q/ha) followed by plots caged with one *A. cerana*

honeybee colony (11.3 q/ha), whereas plots caged without bees (exclusion of pollinators) gave the lowest seed yield (10 q/ha).

Rafid (1992) found in cucumber that the fruit weight was greater (2.69 kg/plant) in honeybee-pollinated plants than in self-pollinated ones (2.03 kg/plant).

Free (1993) who also reported higher seed weight /1000 heads in bee pollinated plots (13.8g) compared to only 0.2g in without insect pollination (WIP).

Benedek *et al.* (1994) reported that lack of bee visitation reduces the fruit set in both self-sterile and self-fertile plum cultivars.

Goodman and Williams (1994) reported that berseem clover flowers have a simple valvular type mechanism of pollination. Honeybees act as tripping agents for better seed setting which improves seed yield. In a study in Victoria, Australia, found that honeybees comprised up to 88% of all insects visiting white clover plants. Exclusion of honeybees has been shown to reduce seed yield significantly, with seed yields of 0.52 g/m<sup>2</sup> and 22.38 g/m<sup>2</sup> between excluded and non-excluded sites, respectively (Dixit *et al.*, 1989).

Goodman and Williams (1994) reported that the presence of honeybees increased white clover seed yield to 168 kg/ha compared to 5.5 kg/ha for caged plots where honeybee activity was largely excluded.

Goodman and Williams (1994) found that seed yield increased by 22% in open pollinated plots by placing three honeybee hives/ha as compared to caged plots without honeybees, which indicates the importance of honeybees as pollinators in Berseem clover seed setting efficacy and yield.

Chand and Singh (1995) observed mustard plots that remained without access to pollinators (caged without any pollinators) had the lowest yield (966 kg/ha). Plots having free access to all the pollinators showed the maximum yield (1620 kg/ha) followed by plots having honeybees (1160 kg/ha). Thus honeybees alone contributed 20% increase in the yield of mustard crop.

Abrol (1996) reported that in sunflower, seed set and yield parameters were higher on plots caged with bees than on open pollinated plants. Results were much lower on plants caged to exclude insects.

Kumar *et al.* (1997) reported that honeybee pollination resulted in higher fruit set in coconut. A 123 per cent increase in fruit set was observed in open pollination over without insect pollination treatment.

Isham and Eisikowitch (1998) reported that avocado depend on honeybees for fruit set. A significant initial fruit set required at least five bees per tree during the stage of flowering.

Rana *et al.* (1998) found that significant increase in per cent fruit set from 32.48 to 53.55 with increase in *Apis mellifera* colonies (2 and 4 colonies) was noticed in apple orchards having adequate pollinator proportion.

Gingras *et al.* (1999) reported that single visit by a bee to a flower was sufficient to induce fruiting on cucumber, Flowers that had the greatest number of visits and highest cumulative duration of visits also had the greatest fruit set.

Malaviya *et al.* (1999) also reported 83.5-84.7 per cent decline in diploid and 60.6-78.4 per cent for tetraploid lines in WIP as compared to open pollination. Caging with *A. florea* improved seed set marginally, while caging with *A. mellifera* recorded lower seed set loss (18.7- 37.8%).

Application of Bee-Q @ 12.50 and 15.00 g/l resulted in higher yield (19.56 and 19.45 t/ha respectively), maximum good fruits, minimum malformed fruits and higher size and weight in watermelon (Sattigi *et al.*, 2001a). Spraying of Bee-Q (12.5 g/l) and bee-here (4 ml/l) on sesamum increased bee visitation on sprayed crop up to 5th day, resulted in significantly higher yield at Dharwad (Patil, 1999 and Patil *et al.*, 2000).

Application of Bee-Q (12.50 g/l) on niger increased the number of seeds/capsule (ranged from 24.41 to 29.26) and oil content (38.10%) as compared to control (Sattigi *et al.*, 2001b).

Priti *et al.* (2001) reported that fruit set was 81 per cent and 56.55 per cent, siliqua length 13.2 and 6.8 cm, number of seeds per pod was 8.6 and 4.8 and seed weight 1.5 and 0.9 grams in bee pollinated and self-pollinated radish, respectively.

Kapila *et al.* (2002) reported that open pollinated plants of radish and greater number of grains per siliqua, seed yield per plant, 100 seed weight, seed set. And root length than the caged plants, indicating the positive effects of insect pollination on seed production and quality.

Ahmed and Rehman (2002) observed in mustard that the number of siliqua per plant was significantly higher in OP (189.60-190.24) over PWI (120.93-120.69). Thapa (2006) found in Indian mustard about 11 per cent increased in pod setting.

Ahmed and Rehman (2002) found that the number of seeds per siliqua (12.04-12.60) per cent higher than PWI in the two varieties of rape seed.

Sharma and Singh (2003) found that seed yield in open pollinated plants ranged from 19.6-70.5 seeds per flower head compared to only 0.3-0.6 seeds where insects were excluded (caged).

Abel *et al.* (2003) observed that in *Brassica rapa* accession PI 392025 and *Brassica napus* accession PI 469944, produced significantly more seeds when pollinated by *Osmia lanigera* sub sp. *Lignaria* than *Osmia cornifrons* Radoszkowski.

Roy *et al.* (2005) found that for caged Berseem clover where pollinators were excluded, seed setting was reduced by 12 to 99% compared to open pollination across all varieties, even in self-compatible lines.

Roy *et al.* (2005) found a reduction of 12.3-99.2 per cent in WIP compared to open pollinated condition.

Cecen *et al.* (2007) reported the highest seed yield of white clover, *Trifolium repens* L. was found in the caged honeybee treatment (46.2 kg/ha) followed by caged bumblebees (37.3kg/ha), open-pollinated (25.7kg/ha) and pollinator excluded (1.37kg/ha).

These findings are amply supported by the work of Singh *et al.*, (2012) who reported higher seed setting under open pollination condition (62.46%) and bee pollination with 4-frame, *A. mellifera* colony (61.01%) and the lowest (40.33%) in without insect pollination.

Singh *et al.* (2012) also reported higher per plot (18 m<sup>2</sup>) seed yield in plots caged with *Apis mellifera* colony (8-frame) as compared to plots caged without insect pollination. Honey bees are reported to be the primary and absolutely essential pollinators of Egyptian clover, visiting it avidly for nectar and pollen and significantly increasing seed yield.

Singh *et al.* (2012) reported that 1000 seed weight (2.47g) in plots caged with *A. mellifera* colony compared to without insect pollination (2.22g).

Singh *et al.* (2012) recorded almost similar but higher seed setting under open pollination condition (62.46%) and bee pollination with 4-frame *A. mellifera* colony (61.01%) and the lowest (40.33%) in without insect pollination. Similarly, many studies reported higher seed set in bee pollination than open pollination.

Bakheit (1989) found highest seed setting in BP with *A. mellifera* (51.9%) followed by hand pollination (17.1%) and least in caged conditions (0.96%). Like in the present studies, many researchers have tried various modes of pollination (Chowdhury *et al.*, 1966; Narayanan *et al.*, 1961; Singh *et al.*, 2012) and accepted the need of honey bees to increase seed yield (McGregor, 1976; Free, 1960). But there is a clear lack of efforts on utilizing various densities of bee populations and that may be the reason of lower seed set recorded by these workers. However, these studies clearly indicate to the highest benefits of saturated pollination where maximum (81.4%) seed setting was recorded.

Studies reported higher seed yields in bee pollination (BP-4f) (130.67g) and least (60.50g) in plots caged without insect pollination (Singh *et al.*, 2012). Similarly, Narayanan *et al.*, (1961) reported higher (1958 and 2671 seeds/100 flower heads) yield in two plots compared to only 27 to 28 seeds with automatic self-pollination in cages during 1957 and also during 1960 at 4360 and 64 to 43 with WIP.

Singh *et al.* (2012) do not support present study who reported lower 1000 – seed weight (2.47g) in plots caged with *A. mellifera* colony compared to without insect pollination (2.22g). The mean data of no. of siliqua per plant showed maximum 186.44 in plots having free access to all the pollinators (OP) followed by 154.82 in BP. The lowest *i.e.* 133.38 number of siliqua/ plant were recorded in PE.

Singh *et al.* (2012) who reported higher seed setting under open pollination condition (62.46%) and bee pollination with 4-frame, *A. mellifera* colony (61.01%) and the lowest (40.33%) in without insect pollination.

The study thus, clearly indicated the immense value of bee pollination in achieving higher seed yield in Egyptian clover. The previous studies reported higher seed yields in bee pollination (BP-4f) (130.67g) and least (60.50g) in plots caged without insect pollination (Singh *et al.*, 2012).

Musa *et al.* (2013) showed that pollination activity of *A. mellifera* caused an increase in mean weight of pods per plant and number of seeds per pod of cowpea exposed to open-pollination. The mean number of seeds per pod (11.15) in plants pollinated by *A. mellifera* (open-pollinated) was significantly higher ( $p < 0.05$ ) than a mean of 9.53 obtained in plants not pollinated, showing a percentage increase of 17.0%. The mean number of empty pods was higher in the non-pollinated as compared to the pollinated cowpea. The installation of hives of *A. mellifera* close to *V. unguiculata* fields could therefore be recommended to improve cowpea pod and seed production in the study area.

Sanas *et al.* (2014) also found that *A. mellifera* increased the number of seed per pod (23.27%) in mustard under Konkan condition of Maharashtra.

Sanas *et al.* (2014), also reported that mustard plot gave maximum seed yield (963.45 Kg/ha) pollinated by free access to all pollinators and lowest yield (602.52 Kg/ha) in pollination without insect. Whereas plots gave yield (763.75 Kg/ha) pollinated by honeybees (*A. cerana indica* Fab).

Jat *et al.*, (2014b) observed that maximum seed yield (2662.3 seeds) was observed in honey bee pollination with 8 combs followed by 4 combs (2373.8 seeds), open pollination (2316.3 seeds) and 2 combs (2235.5 seeds). Still lower yield of (2103.0 seeds) was found in hand pollination treatment that was significantly higher than that without insect pollinator's treatment (1114.2 seeds).

Rodet *et al.* (1998), Rao and Stephen (2009), Goodwin *et al.*, (2011) and Latif *et al.*, (2014) reported for other legume species that employing honeybees as pollinators in Berseem clover crops can significantly increase seed yield through increased seed pollination and therefore seed setting. Similar results of increased seed yields due to honeybee visits and their activity have also been reported by Dixit *et al.*, (1989), Martiniello *et al.*, (2003), Hepburn and Radloff (2011), El-Naby *et al.*, (2012) and Jat *et al.*, (2014b).

Jat *et al.* (2014b) observed that Minimum 1000-seed weight was in without insect pollination (2.64 g). Whereas the seed weight of bee pollination four frame (3.30) Hand Pollination (3.20) bee pollination two frame (3.17) and open pollination (3.03), the heaviest seeds were recorded in bee pollination eight frame (3.62 g/1000 grains).

Bondok *et al.* (2016) found that Maximum Egyptian clover seed yield (2346.6 seeds/40 head) was observed with honey bee pollination six combs followed by four combs (2250.0 seeds) and two combs (1826.6 seeds). Still the lowest seed yield was found in field without insect pollinators.

Bondok *et al.* (2016) who founded that minimum 1000–seed weight with without insect pollination was observed (2.56 g). The 1000 seed weight of bee Pollination–four Frame, six Frame and, bee Pollination two Frame were 3.56, 3.30, 3.80 g, respectively.

Zidan *et al.* (2019) reported that the free pollination treatment produced the highest mean number of seeds per head followed by the caged with honeybees followed by the caged without honeybees treatment with average (48.15, 43.85 and 13.20 seeds /head) and (51.10, 46.10 and 10.85 seeds /head) during 2017 and 2018 seasons, respectively.

Zidan *et al.* (2019) reported that the caged plants without honeybees produced the low weight of 1000 seed. Whereas the non-caged treatment produced the highest weights with an average (2.75 and 2.55 g) and (3.50 and 3.90 g) during both seasons, respectively.

Hosamani *et al.* (2020), reported that open pollinated crop was sprayed with cacambe (10%) recorded significantly higher net plot (4 m x 4 m) yield of 1.42 kg/plot which accounted for increase to the tune of 37.86 and 125.40 per cent over open pollinated plot without spray and caged plot without bees, respectively. Same time it was on par with open pollinated crop sprayed with jaggery solution (10%) (1.41 kg/plot), followed by Bee-Q (1.25%) (1.37 kg/plot) and cage with bee box (1.15 kg/plot). The least net plot yield was received in open pollination and cage without bees (1.03 kg/plot) and (0.63 kg/plot).

## Chapter III

# MATERIALS AND METHODS

This chapter deals with a concise description of the location of the study area, experimental details, observation recorded, methodology, and techniques employed for analysis of observations during the course of the investigation.

The present investigation entitled, “**To study the pollination behaviour and effect of honey bee pollination on quantitative and qualitative parameters of Egyptian Clover *Trifolium alexandrinum* L.**” was carried in the assigned experimental field of Egyptian clover and the apiary at RVSKVV-Krishi Vigyan Kendra, Morena, Madhya Pradesh, during 2020-21. The Krishi Vigyan Kendra is situated at 26.47°N latitude, 77.98°E longitude, and 177m AMSL. The mean annual minimum and a maximum temperature range from 2 to 50°C. The average annual precipitation received is 750 mm.

### **To study the pollinator fauna on Egyptian clover, *Trifolium alexandrinum* L.**

The study was conducted during crop season *rabi* 2020-21. The experimental field was kept free from any pesticide spray during the flowering period. The direct count observation method was used to survey certain insects that visited and occurred on plants. Observations were recorded for different groups of pollinators visiting the Egyptian clover crop field during flowering at 0800, 1000, 1200 and 1600 hours for five minutes in each square meter area from three spots during the peak flowering period in the Egyptian clover crop field. Unknown insects were collected with suitable collection procedures and later were identified with the help of a major advisor and members. The observed data were averaged time-wise and group-wise to infer the pollinator fauna as well as the dominance (in percent value) of a particular group. The dominance percent was calculated by dividing the cent percent population by the total number of genera observed and then multiplying it with the number of species observed in a particular insect order group.

**To study the effectiveness of different bee attractants to attract the bees in Egyptian clover.**

**Details of the experiment:**

Design	: RBD
Crop	: Berseem
Variety	: Bundel berseem 1
Plot size	: 6.0 m x 3.0 m
No. of replication	3
No. of treatments	: 8 (including control)
Fertilizer doses	: 20:80-90:30-40 kg (N:P:K/ha.)

The experiment was laid out in Randomized Block Design (RBD) with eight treatments replicated thrice. The treatment details are as follows:

- T1: Open pollination Without Treatment (Control)
- T2: Open pollination with Sugar solution @ 10%
- T3: Open pollination with Coriander @ 1.25 %
- T4: Open pollination with Jaggery @ 10%
- T5: Open pollination with Cacambe @ (10%).
- T6: Open pollination with Onion Solution @ 10%
- T7: Open pollination with Sugarcane juice @ 10%
- T8: Open pollination with Bee-Q @ 1.25 %

All the treatments were imposed at the 10 percent flowering stage of the crop. The crop was protected from various pests and diseases, but no insecticides were used during the flowering period. Recommended agronomical package of practices were followed for raising good seed production plot. The attractants will be sprayed three times at 10 days interval starting from 10 percent flowering. In each plot one-meter square area was randomly selected and marked and number of *A. mellifera* pollinator bees visited the flowers in the selected area per five minutes was recorded. The observations were taken at five different time slots viz. 0800, 1000, 1200, 1400 and 1600 hours during the flowering period and observed data was averaged on day basis. Such observations were made a day before the spray and 1, 3, and 5 days after I<sup>st</sup>, II<sup>nd</sup> and III<sup>rd</sup> spray.

**To study the Effect of bee pollination on quality and quantity parameters of Egyptian clover.**

**Details of the experiment:**

Design	: RBD
Crop	: Berseem
Variety	: Bundel berseem 1
Plot size	: 6.0 m x 3.0 m
No. of replication	3
No. of treatments	: 10 (including control)
Fertilizer doses	: 20:80-90:30-40 kg (N:P:K/ha.)

The crop sowing practices were initiated during the first fortnight of the month of November 2020 following all the recommended agronomic practices. The field was kept unsprayed by pesticides throughout the crop season. There were ten treatments which were categorized into (OP) Open Pollination (T1 to T8), Caged with bee or (BP) Bee Pollination (T9), and Caged without bees or (WIP) Without Insect Pollination (T10).

The experiment was laid out in Randomized Block Design (RBD) with ten treatments replicated thrice into 30 plots. The treatment details are as given below:

**T1-T8- Open pollination treated with bee attractants (OP):** These plots were not caged and remained exposed/open for access to pollination by all agencies at all times. All the treatments were imposed at the 10 percent flowering stage of the crop except the T1 plot. The spraying of the treatments was done three times during the flowering season of the crop from T2 to T8. The first spray will be done at the 10 percent flowering stage of the Egyptian clover crop. The second spray was done 10 days after the first spray followed by the third spray after 10 days of the second spray. The treatment name, their concentrations, and formulations are given below:

- T1: Open pollination without any treatment
- T2: Open pollination with Sugar solution @ 10%
- T3: Open pollination with Coriander @ 1.25 %
- T4: Open pollination with Jaggery @ 10%

T5: Open pollination with Cacambe @ (10%).

T6: Open pollination with Onion Solution @ 10%

T7: Open pollination with Sugarcane juice @ 10%

T8: Open pollination with Bee-Q @ 1.25 %

**T9- Caged with Bees or Bee pollination (BP):** In the treatment of bee pollination, the plots were caged before the initiation of flowering during the night to eliminate natural pollination. The crop was caged at the beginning of flowering and sprayed with chlorpyrifos 20 EC to kill all the insects inside the cage to exclude their contribution towards pollination. After the waiting period of insecticide, a bee colony with *Apis mellifera* L. in a standard deep LT wooden hive box was used and kept just at the start of flowering, not later than 10 percent flowering till completion of flowering. For this purpose, special beehive boxes with back and front entrances were kept open in one boundary of the cage to facilitate the bees to move in and out of the cage. The colony strength was according to the sufficient availability of the flora. The colony was regularly provided with water. The fine nylon mosquito net cages of size 4x3x2 m, sufficient to allow unhindered movement of honey bees inside the cage, were erected on a standing crop before the initiation of flowering. The nets were got stitched using nylon cloth of 16-mesh size sufficient to exclude the entry of insects in the cage but did not hinder other physical factors. Iron pipes are used for making the cage to hold the nylon net in erected position and tightened with the help of cords. Lower edges of the mosquito net were touched to the ground and sufficiently pressed under the soil so as to exclude hovering insects away from the covered plants. A zipped entry was kept at one corner of the net to allow manual entry for data recording and other operations. Care was taken to immediately close the zip of the cage after entry into the cage and exit of the cage. The bee colony was removed when all the floral bloom was exhausted. The cage was also removed after the completion of flowering.

**T10- Caged Without Bees or Without insect pollination (WIP):** In WIP or caged without bees (CWB), the plants in the plot were caged in a nylon net (4x3x2m) of 16 mm mesh size as explained above in bee pollination. The crop was sprayed with chlorpyrifos 20 EC to kill all the insects inside the cage to prevent their contribution towards pollination. However, in WIP, no honey bee

colony was placed inside. The cage net prevented pollination by any insect or other pollinators.

### **Qualitative and Quantitative parameters**

In order to study the effect of bee pollination in enhancing the productivity and quality of Egyptian clover, the following quantitative and qualitative parameters were recorded from each treatment. Observations were recorded on five randomly selected (avoiding edges) plants per treatment for five different characters *viz.* number of florets per raceme, number of seeds per raceme, seed setting (%), number of seeds per 50 Raceme (seed yield), and 1000 seeds weight (g) in each replication. The effective methods of observations for different characters are described below:

**Recording of observations:** Flower heads or racemes from randomly selected plants were tagged in each replication of different treatments. At maturity, they were manually harvested. The following quantitative and qualitative yield parameters were recorded from these racemes.

**Number of florets per raceme:** After harvesting, five flower heads (racemes) were randomly selected from each replication of the treatment. Number of florets per raceme was counted and their mean was calculated.

**Number of seeds per raceme:** After counting the number of florets from the above-selected racemes, the seeds were removed and counted carefully. The number of seeds per raceme was also counted and the mean seed number was determined.

**Seeds setting (%):** From randomly selected and marked five racemes, the number of florets per raceme was counted and at the harvesting stage, the number of seeds per raceme was counted. The seed setting percent was calculated by the given formula:

$$\text{Seed setting (\%)} = \frac{\text{No.of seeds per raceme}}{\text{No.of florets per raceme}} \times 100$$

**Number of seeds (seed yield):** After maturity, the seeds were separated by threshing the raceme and the total number of seeds obtained from the threshing of 50 racemes was counted with the help of seed counter (Numigral) and weight was recorded by using an electronic balance which expressed in grams per

plant. The seed yield obtained from the plots was later converted into a q per ha basis. All the data were subjected to statistical analysis after suitable transformations.

( $\sqrt{x+1}$  for numbers and Arcsine for percent)

**1000-seed weight:** A thousand seeds from each replication of individual treatments were counted and weighed with the help of an electronic weighing machine to get 1000-seed weight in grams.

### **Statistical analysis**

The collected data were cleared & stored into Microsoft Excel spread sheets after the completion of data collection work from the study areas for further analysis. Summarized data was presented in the form of tables and figures.

For the effectiveness of bee attractants, data were transformed using the square root transformation as per the method described by Gomez and Gomez (1986).

For the analysis of the effect of bee pollination on qualitative and quantitative characters, the data were tested for normality and no data transformations were required. The data were then subjected to statistical analysis by adopting appropriate method of analysis of variance as described by Fisher (1958). Wherever, variance ratio (calculated 'F' values) was found significant, critical difference (C.D.) values were computed by following formula for making comparison between the treatments.

$$C.D. = \sqrt{\frac{\text{error variance}}{n}} \times \sqrt{2} \times t$$

where, n = The number of observations averaged

Error variance = mean sum of squares due to error and 't' is the value from t-table at 5% level of significance and at error d.f.

## CHAPTER IV

### RESULTS AND DISCUSSION

The results and discussion of the present investigation entitled “**To study the pollination behaviour and effect of honey bee pollination on quantitative and qualitative parameters of Egyptian Clover *Trifolium alexandrinum* L.**” are presented in this chapter.

#### **To study the pollinator fauna of Egyptian clover.**

Studies on the visit of pollinators' fauna in the field of Egyptian clover revealed that about seventeen species of insects were observed and are listed in descending order in table 1 to give a general picture of the arthropod specimens that have been identified. This list includes all the insects that have visited the field during the observation whether they are harmful or beneficial to the crop. This is because the experiment was conducted without the application of any single chemical spray as this can be harmful to other micro fauna and pollinators present in the berseem ecosystem. However, listed arthropods represent 18 genera of Arthropods belong to 13 families and 7 orders. The identified insect species that are listed in this table were classified into Phytophagous, Predator, and Nectarivore according to their behaviour.

Order Hymenoptera was observed to be the most dominant and includes the most active pollinators with 44.44 percent dominance followed by Lepidopterans, Dipterans, Coleopterans, Hemipterans, Neuropterans, and Odonata which were found at 16.66, 11.11, 11.11, 5.55, 5.55, and 5.55 percent dominance, respectively (Fig. 1). Bee pollinators visiting during flowering time includes *Apis dorsata*, *Apis mellifera*, *Apis cerana indica* and *Apis florea*. The study also shows that *Apis dorsata* mainly targets the upper region of crop canopy, while *Apis mellifera*, *Apis cerana indica* and *Apis florea* forages in the flowers of lower region of crop canopy. Also *Apis dorsata* spent lesser time per flower and visited more number of flowers per minute than *Apis mellifera* and *Apis florea* visits. Thus, it shows that *A. dorsata* is more effective and predominant pollinators with good frequency in berseem followed by *Apis mellifera* and *Apis florea*. However, this may depend from place to place and

on the presence of various bee populations in the proximity of the crop under natural conditions.

The study further reveals that the pollinators start their activity from 0800 hours and remains active until 1700 hours. Most of the pollinators' remains active in the morning hours. The peak activity of pollinators was observed approximately at 1000 hours followed by drop in the activity during noon time. Even though bee activity again increases in the afternoon near about 1200 hours and those bees are assumed to be nectar collectors rather than pollen gatherers. Later the activity of pollinators in the evening hours was observed to be less.

These data are in line with those of Mabrouk and Mahbob (2017), who recorded in the Egyptian clover field twenty-two insect species, including five species of pests, five species of natural enemies, and twelve pollinators (*Phyllanthus abdelkader*, *Polistes gallica*, *Vespa orientalis*, *Eumenus maxillosa*, *Megachile uniformis*, *Megachile submucida*, *Megachile muntusemina*, *Osmia* spp., *Chalcidoma siculum*, *Apis mellifera* L. *Andrena* spp. and *Ocnera hispida*). Wagan *et al.*, (2015) stated that insect pests on the berseem crop, were: aphid, leaf miner, dusky bug, thrips, whitefly, cricket, and cutworm, respectively. Whereas the natural enemies were; rove beetles, ladybird beetle, ant, spider, big-eyed, and green lacewing. Hameed *et al.*, (2016) revealed that armyworm, *Spodoptera exigua*, aphid (*Sitobian avenae* L.) and leaf miner (*Aproaerema modicella*) are important pests of berseem while the *Coccinellids* and a *Syrphid* fly are important predators and honeybee are important pollinators of berseem.

Hesain (2013) recorded eleven species of bees and other insects on the flower of the clover, which are four species from hymenoptera, (*Apis mellifera*, *Megachil* sp., *Halictus* sp. and *Anderena* sp.), three species of diptera were (*Metasyrphus taeniops*, *Eristalis anaeus* and *Episyphus balteatus*) and four species of *Lepidoptera* it were (*Lycaeides Melissa* (alpime), *Autograph gamma* L., *Colias croceus* L., and *Artogeia rapa*). The major predators and pollinators include the *Coccinellidae*, a *syrphid* fly and honey bees. (Shamel, 1956; Narayanan *et al.*, 1961; Hassanein, 1953).

**Table 1: List of pollinators' fauna observed and collected from Egyptian clover by direct count method during 2020-21 flowering season.**

Insect Order	Family	Common name	Scientific name	Status
Hemiptera	Pentatomidae	Green stink bug	<i>Nezara vridula</i> L.	Phyto.
Neuroptera	Chrysopidae	Green lacewing	<i>Chrysoperla carnea</i> (Steph.)	Nect. Pre.
Coleoptera	Coccinellidae	Lady bird beetle	<i>Coccinella septumpunctata</i> L.	Pre.
	Curculionidae	Egyptian alfalfa weevil	<i>Hypera brunneipennis</i> Boh.	Pre.
Odonata	Aeshnidae	Dragon fly	<i>Hemianax ephippiger</i> Burmister	Pre.
Lepidoptera	Lycaenidae	Pea blue butterfly	<i>Lampides boeticus</i> L.	Nect. & Phyto.
	Noctuidae	Cotton leaf worm	<i>Spodoptera littoralis</i> Boisduval	Nect. & Phyto.
		Beet armyworm	<i>Spodoptera exigua</i> Hubner	Nect. & Phyto.
Hymenoptera	Apidae	Honeybee	<i>Apis dorsata</i> L.	Nect.
			<i>Apis mellifera</i> L.	Nect.
			<i>Apis cerana indica</i>	Nect.
			<i>Apis florea</i>	Nect.
	Megachilidae	Alfalfa leafcutter bee	<i>Megachile</i> sp.	Nect.
	Vespidae	Oriental hornet	<i>Vespa orientalis</i> L.	Nect. & Pre.
		Paper wasp	<i>Polistes gallicus</i> L.	Nect.
Yellow jacket		<i>Vespula squamosa</i> D.	Nect.	
Diptera	Muscidae	Orientalis house fly	<i>Musca domestica</i> (Macq.)	Nect.
	Syrphidae	Hover fly	<i>Syrphus</i> spp.	Nect. & Pre.

**Nect:** Nectarivore, insects feed on nectar produced by flowering plants.

**Pre:** Predator, an insect that feeds on other insects or one of their stages

**Phyto:** Phytophagous, an insect feeding on plants or plant material.

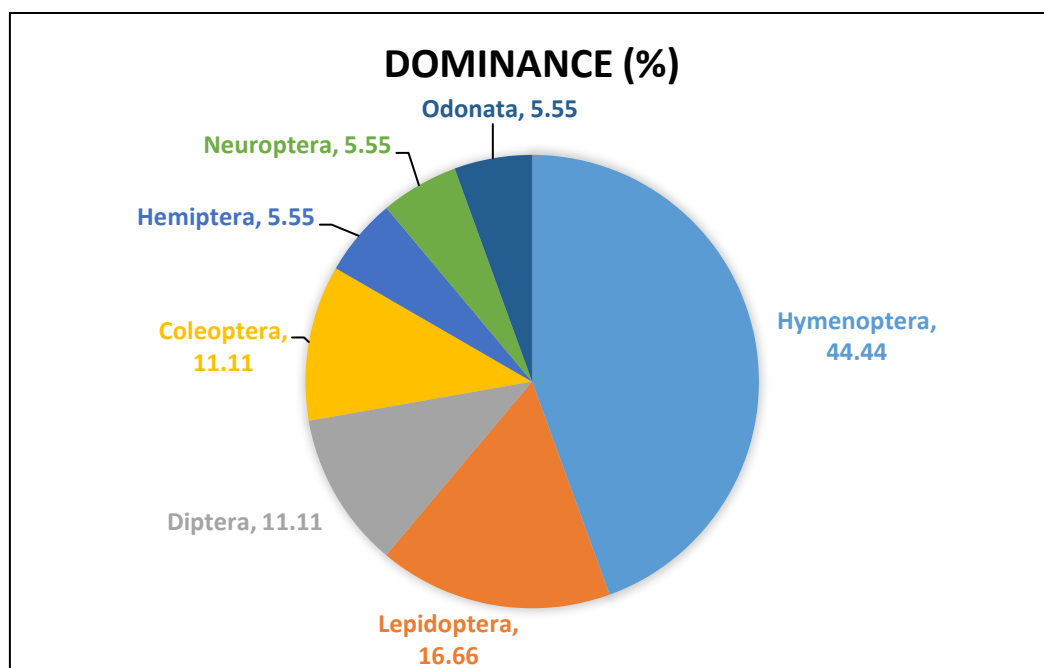
**Nect. & Phyto:** Insects their larvae feed on the plant and the adult feeds on the nectar.

**Nect. & Pre:** Insects, their larvae feed on the insects and the adult often feeds on the nectar

**Table 2: Group wise percent dominance of pollinators' fauna observed from Egyptian clover during 2020-21 flowering season.**

Sr. No.	Insect Order	No of species	Dominance (%)
1	Hymenoptera	8	44.44
2	Lepidoptera	3	16.66
3	Diptera	2	11.11
4	Coleoptera	2	11.11
5	Hemiptera	1	5.55
6	Neuroptera	1	5.55
7	Odonata	1	5.55

**Fig. 1 Dominance of pollinators' fauna of different insect orders in Egyptian clover during 2020-21.**



## **To study the effectiveness of different bee attractants to attract the bees in Egyptian clover.**

Influence of attractants on visitation of *A. mellifera* was studied and the observations on bee visitation to Egyptian clover crop sprayed with different bee attractants at different flowering stages are described and briefly discussed on the basis of number of sprays and flowering stage.

### **First spray**

In order to achieve the proper and efficient pollination and to improve the qualitative and quantitative characteristics of the Egyptian clover, the efficacy of several bee attractant sprays in the Egyptian clover crops was evaluated during the flowering stage with 1<sup>st</sup> spray at 10 percent flowering stage, and the results are listed in Table 3.

A day before spray, the treatments applied to the crop were found to be non-significant. During this bee activity ranges from 18.40 to 18.73 bees/m<sup>2</sup>/5min.

The effectiveness of the treatment on 1 day after spraying with 10 percent Cacambe was found to be significantly most effective and superior treatment in attracting higher number bees *i.e.*, 35.60 bees/m<sup>2</sup>/5min and 1.25 percent Bee-Q attracting 34.53 bees/m<sup>2</sup>/5min being at par with each other followed by sugarcane juice 10 percent attracting 31.80 bees/m<sup>2</sup>/5min which was also found statistically at par with Jaggery 10 percent (31.07 bees/m<sup>2</sup>/5min). The remaining treatments attracted *A. mellifera* visits ranging from 24.87 to 28.07 bees/m<sup>2</sup>/5min which were significantly poor in attracting the visitors as compared to above treatments but attracted a greater number of bees than open pollination without any treatment (21.87 bees/m<sup>2</sup>/5min).

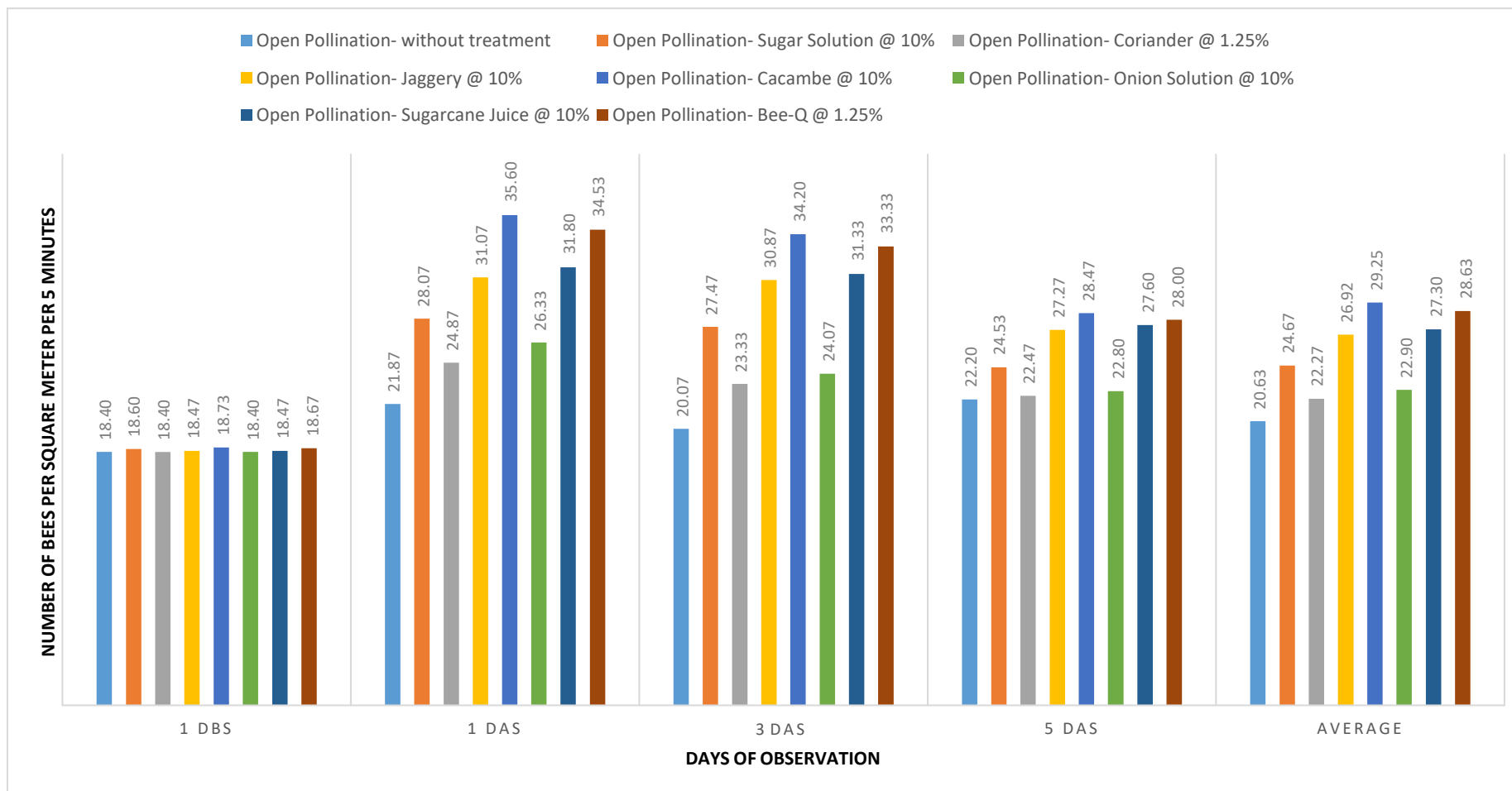
On 3<sup>rd</sup> day after the first spray, the same trend of visit of bees to the treated plots was observed. Cacambe 10 percent (34.20 bees/m<sup>2</sup>/5min) was significantly superior in attracting greater no. of bees followed by 33.33 bees/m<sup>2</sup>/5min in the plot treated with Bee-Q 1.25 percent. Effectiveness of the next best treatment was 31.33 bees/m<sup>2</sup>/5min visiting the plot treated with Sugarcane Juice 10 percent which was found at par with 30.87 bees/m<sup>2</sup>/5min visiting in the plot treated with Jaggery 10 percent. The lowest number of bees

**Table 3: Effectiveness of different bee attractants to attract the bees in Egyptian clover in 1<sup>st</sup> spray.**

Treatments	Bee visits/m <sup>2</sup> /5 min (1st spray at 10% flowering)				
	1 DBS	1 DAS	3 DAS	5 DAS	Mean
T1- Open Pollination- without treatment	18.40 (4.29)*	21.87 (4.68)	20.07 (4.48)	22.20 (4.69)	20.63 (4.54)
T2- Open Pollination- Sugar Solution @ 10%	18.60 (4.31)	28.07 (5.30)	27.47 (5.24)	24.53 (4.95)	24.67 (4.97)
T3- Open Pollination- Coriander @ 1.25%	18.40 (4.29)	24.87 (4.99)	23.33 (4.83)	22.47 (4.74)	22.27 (4.72)
T4- Open Pollination- Jaggery @ 10%	18.47 (4.30)	31.07 (5.57)	30.87 (5.56)	27.27 (5.22)	26.92 (5.19)
T5- Open Pollination- Cacambe @ 10%	18.73 (4.33)	35.60 (5.97)	34.20 (5.85)	28.47 (5.34)	29.25 (5.41)
T6- Open Pollination- Onion Solution @ 10%	18.40 (4.29)	26.33 (5.13)	24.07 (4.91)	22.80 (4.78)	22.90 (4.79)
T7- Open Pollination- Sugarcane Juice @ 10%	18.47 (4.30)	31.80 (5.64)	31.33 (5.60)	27.60 (5.25)	27.30 (5.23)
T8- Open Pollination- Bee-Q @ 1.25%	18.67 (4.32)	34.53 (5.88)	33.33 (5.77)	28.00 (5.29)	28.63 (5.35)
<b>C.D.**</b>	-	0.094	0.059	0.330	0.095

DBS – Day Before Spray; DAS – Day After Spray \*square root transformed values \*\*significant at 5% level of significance

**Figure 2: Graph showing the effectiveness of different bee attractants to attract the bees in Egyptian clover in 1<sup>st</sup> spray.**



were observed in treatments with open pollination without any treatment (20.07 bees/m<sup>2</sup>/5min).

On 5th day after first spray, treatment Cacambe 10 percent and Bee-Q 1.25 percent were found to be effective in attracting highest number of bees, *i.e.* 28.47 and 28.00 bees/m<sup>2</sup>/5min respectively, being the superior treatment over other treatments showing the statistically same effects. The next better followed treatment was Sugarcane juice attracting 27.60 bees/m<sup>2</sup>/5min which was found on par with Jaggery 10 percent and Sugar solution 10 percent (27.27 and 24.53 bees/m<sup>2</sup>/5min respectively) treatments. However, the least effective treatment was untreated control, thus attracting 22.20 bees/m<sup>2</sup>/5min.

On an average Cacambe 10 percent showed relatively highest bee activities with 29.25 bees/m<sup>2</sup>/5min which was found to be having statistically same effects with the plot treated with Bee-Q 1.25 percent showing 28.63 bees/m<sup>2</sup>/5min, followed by sugarcane juice 10 percent with 27.30 bees/m<sup>2</sup>/5min and jaggery solution 10 percent with 26.92 bees/m<sup>2</sup>/5min being at par. The next significant effects were showed by Sugar solution 10 percent (24.67 bees/m<sup>2</sup>/5min) followed by Onion solution 10 percent and Coriander 1.25 percent 22.90 and 22.27 bees/m<sup>2</sup>/5min respectively. The least average bee activities were observed in untreated control plot (20.63 bees/m<sup>2</sup>/5min).

### **Second spray**

The effectiveness of several bee attractants sprays on Egyptian clover crop during flowering phase at 2<sup>nd</sup> spray after 10 days from the 1<sup>st</sup> spray to attract *A. mellifera* for proper pollination and to enhance the qualitative and quantitative characters of Egyptian clover was evaluated, and the results are listed in Table 4.

A day before second spray, bee activity of *A. mellifera* was observed ranging from 20.93 to 25.53 bees/m<sup>2</sup>/5min. The effectiveness of the treatments Cacambe 10 percent and Bee-Q 1.25 percent applied in the crop were found at par (25.53 and 25.07 bees/m<sup>2</sup>/5min as the flowering was at near its peak. The least influencing treatment observed was untreated control (20.93 bees/m<sup>2</sup>/5min).

One day after second spraying, the treatment with Cacambe 10 percent and Bee-Q 1.25 percent were found significantly superior and statistically same

in attracting a greater no. of bees (37.60 and 36.53 bees/m<sup>2</sup>/5min) followed by sugarcane juice 10 percent (33.80 bees/m<sup>2</sup>/5min) and Jaggery solution 10 percent (33.07 bees/m<sup>2</sup>/5min) being at par and sugar solution 10 percent (30.07 bees/m<sup>2</sup>/5min) was next optimal treatment. Open pollination (without any treatment spray) was recorded to attract the lowest number of bees (24.07 bees/m<sup>2</sup>/5min).

Among different treatments, spraying of Cacambe 10 percent was found to be significant best as compared to other treatments (35.60 bees/m<sup>2</sup>/5min) on the 3<sup>rd</sup> day after second spray and was statistically at par with Bee-Q 1.25 percent (35.20 bees/m<sup>2</sup>/5min) which was followed by Jaggery solution 10 percent (33.27 honey bees/m<sup>2</sup>/5min) and sugarcane juice 10 percent (32.87 honey bees/m<sup>2</sup>/5min). However, the treatment with open pollination, which recorded as 21.87 bees/m<sup>2</sup>/5min found to be lowest bee attracting treatment. On 5th day after second spray Cacambe 10 percent, Bee-Q 1.25 percent, Sugarcane juice 10 percent and Jaggery 10 percent attracted maximum number of bees ranging from 27.67 to 28.87 bees/m<sup>2</sup>/5min, found significantly superior over the other treatments and were on par with each other. The next best treatment were sugar solution 10 percent (24.93 bees/m<sup>2</sup>/5min). However, onion solution 10 percent and coriander 1.25 percent were also found successful in attracting more bees, *i.e.* 23.20 and 22.87 bees/m<sup>2</sup>/5min, over control (21.53 bees/m<sup>2</sup>/5min) which was ineffective in attracting more bees (Table 4).

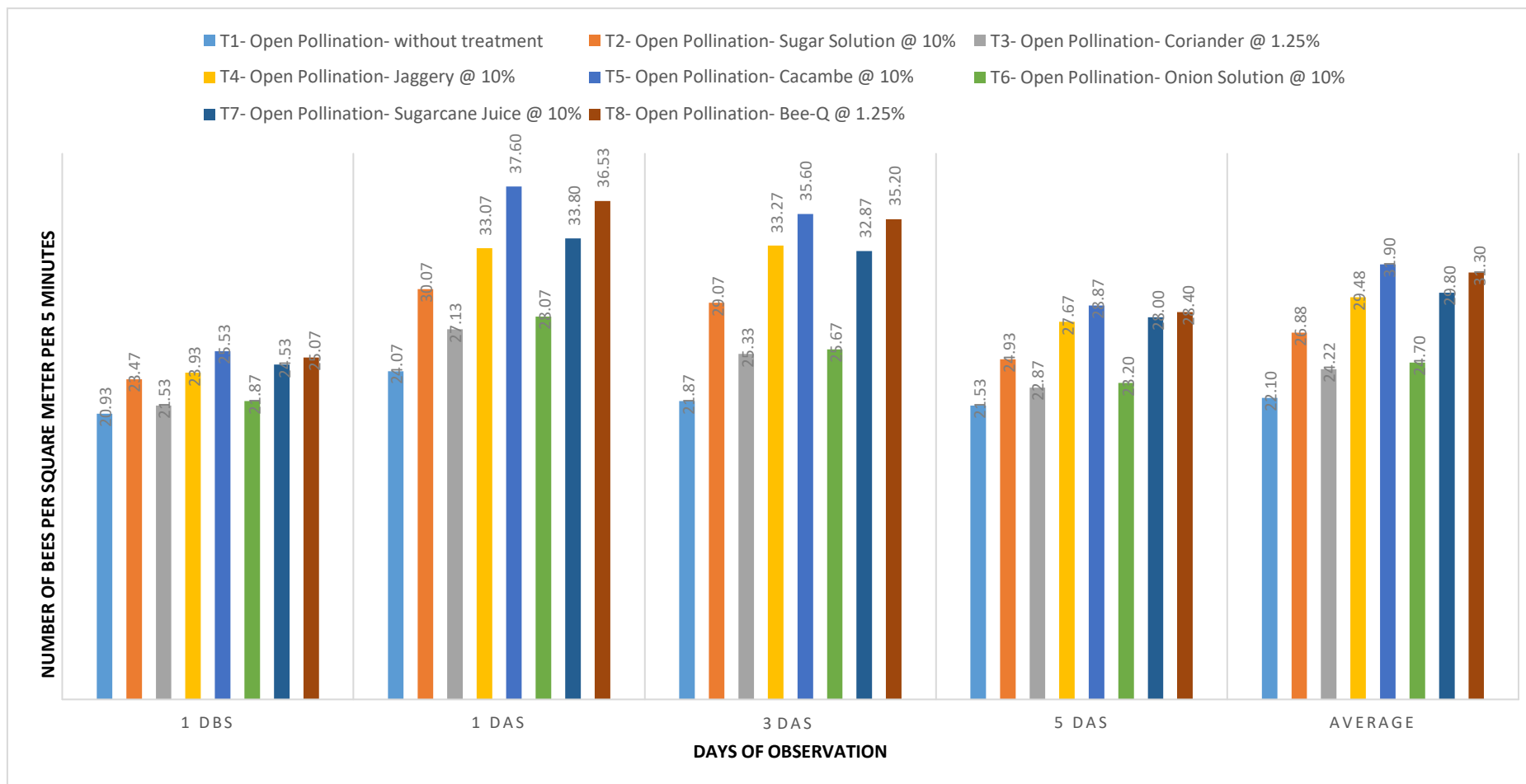
Cacambe 10 percent showed highest bee activities with 31.90 bees/m<sup>2</sup>/5min on average throughout the period of observation on second spray, which was found to have statistically same effects with the plot treated with Bee-Q 1.25 percent showing 31.30 bees/m<sup>2</sup>/5min, followed by sugarcane juice 10% with 29.80 bees/m<sup>2</sup>/5min and jaggary solution 10% with 29.48 bees/m<sup>2</sup>/5min being at par with each other. Sugar solution 10 percent, Onion solution 10 percent, and Coriander 1.25 percent (26.88, 24.70 and 24.22 bees/m<sup>2</sup>/5min, respectively) were the next three significant impacts. The untreated control plot had the lowest average bee activity (22.10 bees/m<sup>2</sup>/5min).

**Table 4: Effectiveness of different bee attractants to attract the bees in Egyptian clover 2<sup>nd</sup> spray.**

Treatments	Bee visits/m <sup>2</sup> /5 min (2 <sup>nd</sup> spray at 10 Days after first spray)				
	1 DBS	1 DAS	3 DAS	5 DAS	Mean
T1- Open Pollination- without treatment	20.93 (4.58)*	24.07 (4.91)	21.87 (4.68)	21.53 (4.63)	22.10 (4.70)
T2- Open Pollination- Sugar Solution @ 10%	23.47 (4.84)	30.07 (5.48)	29.07 (5.39)	24.93 (4.99)	26.88 (5.19)
T3- Open Pollination- Coriander @ 1.25%	21.53 (4.64)	27.13 (5.21)	25.33 (5.03)	22.87 (4.78)	24.22 (4.92)
T4- Open Pollination- Jaggery @ 10%	23.93 (4.89)	33.07 (5.75)	33.27 (5.77)	27.67 (5.26)	29.48 (5.43)
T5- Open Pollination- Cacambe @ 10%	25.53 (5.05)	37.60 (6.13)	35.60 (5.97)	28.87 (5.37)	31.90 (5.65)
T6- Open Pollination- Onion Solution @ 10%	21.87 (4.68)	28.07 (5.30)	25.67 (5.07)	23.20 (4.82)	24.70 (4.97)
T7- Open Pollination- Sugarcane Juice @ 10%	24.53 (4.95)	33.80 (5.81)	32.87 (5.73)	28.00 (5.29)	29.80 (5.46)
T8- Open Pollination- Bee-Q @ 1.25%	25.07 (5.01)	36.53 (6.04)	35.20 (5.93)	28.40 (5.33)	31.30 (5.60)
<b>C.D.**</b>	0.057	0.094	0.080	0.262	0.065

DBS – Day Before Spray; DAS – Day After Spray \*square root transformed values \*\*significant at 5% level of significance

**Figure 3: Graph showing the effectiveness of different bee attractants to attract the bees in Egyptian clover in 2<sup>nd</sup> spray.**



### Third spray

The effectiveness of several bee attractants sprays on Egyptian clover crop during flowering phase at 3<sup>rd</sup> spray after 10 days from the 2<sup>nd</sup> spray to attract *A. mellifera* for proper pollination and to increase the qualitative and quantitative characters of Egyptian clover was evaluated, and the results are listed in Table 5.

A day before third spray, bee activity of *A. mellifera* was observed ranging from 20.07 to 24.73 bees/m<sup>2</sup>/5min. The effectiveness of the treatment Cacambe 10 percent was found 24.73 bees/m<sup>2</sup>/5min as the flowering stage is going to complete. The least influencing treatment observed was untreated control (20.07 bees/m<sup>2</sup>/5min).

One day after third spraying, the treatment with Cacambe 10 percent and Bee-Q 1.25 percent were found significantly superior and statistically same in attracting a greater number of bees (26.80 and 26.67 bees/m<sup>2</sup>/5min respectively) followed by sugarcane juice 10 percent (25.53 bees/m<sup>2</sup>/5min) and Jaggery solution 10 percent (24.67 bees/m<sup>2</sup>/5min). Onion solution 10 percent and coriander 1.25 percent attracting 22.53 and 22.07 bees/m<sup>2</sup>/5min respectively was found at par with Open pollination (without any treatment spray) observing the lowest number of bees (24.07 bees/m<sup>2</sup>/5min).

Amidst different treatments, spraying of Cacambe 10 percent was found to be superior compared to other treatments (24.93 bees/m<sup>2</sup>/5min) on the 3<sup>rd</sup> day after third spray and was statistically at par with Bee-Q 1.25 percent (24.53 bees/m<sup>2</sup>/5min) followed by sugarcane juice 10 percent (22.87 bees/m<sup>2</sup>/5min). However, the treatment with open pollination, which was recorded as 20.20 bees/m<sup>2</sup>/5min found to be lowest bee attracting treatment.

On 5th day after third spray Cacambe 10 percent, Bee-Q 1.25 percent, Sugarcane juice 10 percent, Jaggery 10 percent and sugar solution 10 percent attracted statistically same number of bees ranging from 21.67 to 22.80 bees/m<sup>2</sup>/5min, found significantly over the other treatments. However, coriander 1.25 percent and onion solution 10 percent were also found successful in attracting more bees, *i.e.* 20.47 and 20.33 bees/m<sup>2</sup>/5min, over control (18.53 bees/m<sup>2</sup>/5min) which was ineffective in attracting more bees (Table 5).

Cacambe 10 percent showed relatively highest bee activities with 24.82 bees/m<sup>2</sup>/5min on average throughout the period of observation on third spray, which was found to have statistically same effects with the plot treated with Bee-Q 1.25 percent showing 24.58 bees/m<sup>2</sup>/5min, followed by sugarcane juice 10% with 23.60 bees/m<sup>2</sup>/5min and jaggery solution 10% with 23.13 bees/m<sup>2</sup>/. Onion solution 10 percent and Coriander 1.25 percent were recorded at 21.72 and 21.40 bees/m<sup>2</sup>/5min, respectively. The untreated control plot had the lowest average bee activity (20.17 bees/m<sup>2</sup>/5min).

All these findings and results revealed from the current experiment are in line with the previous studies and research work. The results are followed by Kalmath and Sattigi (2002) who reported that spraying of Cacambe (10 %) and jaggery (10%) attracted maximum number of *A. dorsata* and *Apis mellifera* up to 15 days after first and second spray Bee-Q (1.25%) and sugar solution were the next best attractants. Nidagundi (2004) reported that in the comparison application of several bee attractants and open pollination condition, spraying cacambe 10 percent, Bee-Q 1.25 per cent, and jaggery solution 10 percent had a substantial impact in attracting the higher number of pollinators than open pollination to the flowers of bitter melon. Viraktamath *et al.*, (2002) reported that according to the study on bee attractants on *Cucumis sativa*, two applications of Bee-Q (12.5 gm/lit), Bee-hera (4 ml/lit), and sugar solution (10 percent) on staminate flowers of *Cucumis sativa* enticed a greater number of bees (4.01 to 4.97 bees/flower in 5 minutes) up to 5 days after the first and second sprays collected to unsprayed crop (3.25 to 3.59 honey bees). On the treated crop, more visitations were reported on pistillate flowers.

The present studies are also supported by Manjunath (2003) who reported that spraying of fruit boost and Bee-Q significantly enhanced visitation by *A. dorsata*, *A. cerana*, *A. mellifera* and other pollinators. However, attractants lost their efficacy after 5 days of spraying in sunflower. Manchare *et al.*, (2020) stated that the bee attractants were sprayed twice, first at 10% blooming and again at 50% flowering. According to the results of bee foraging activity, the intensity of *Apis dorsata* increased one day after spraying and decreased seven days later. Spraying bee attractants, such as honey solution 10%, jaggery solution 10%, and molasses 10%, attracted the greatest number of *Apis dorsata*

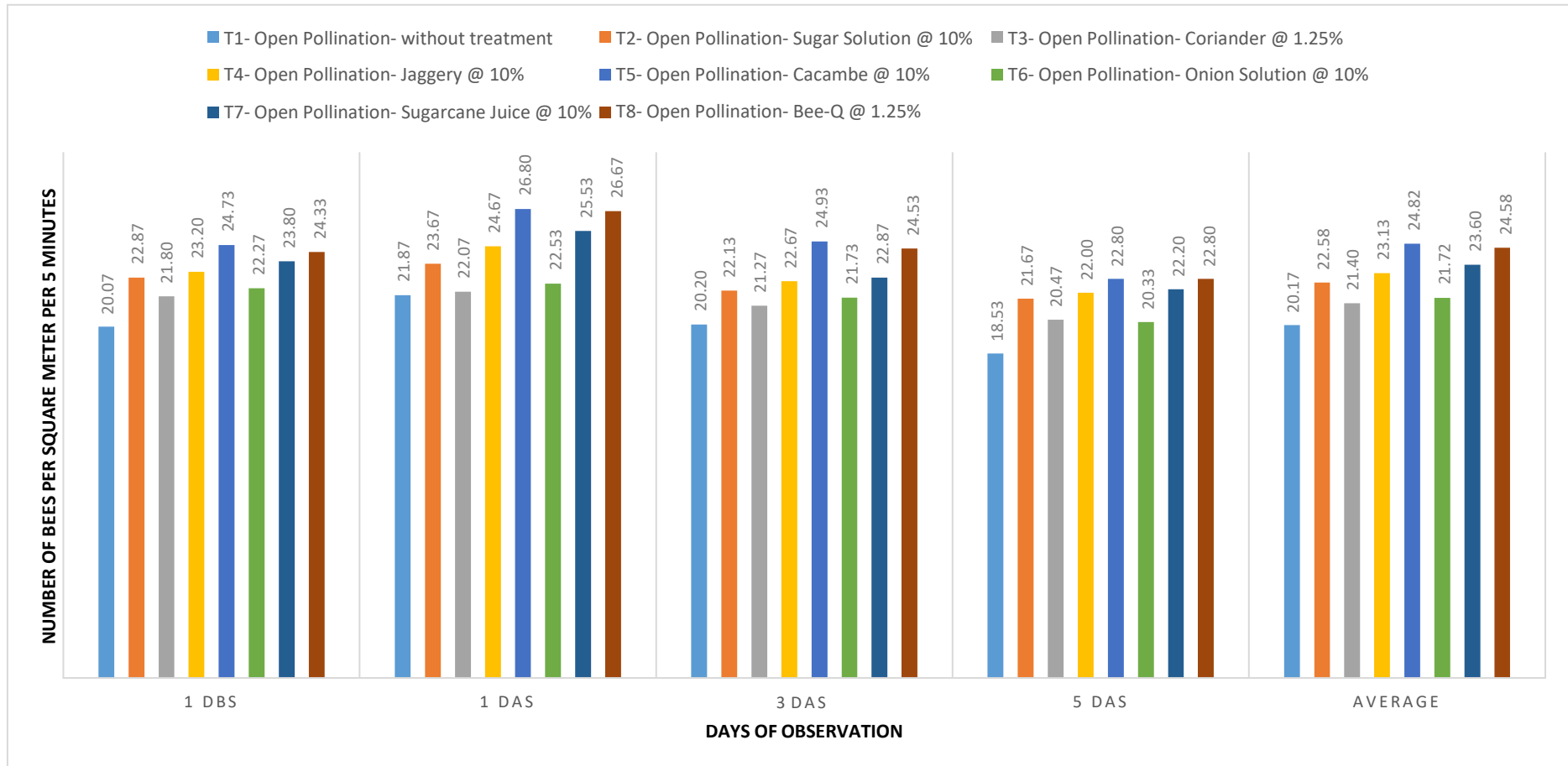
up to the fifth day after the first spray and the seventh day after the second spray. Venkataramgowda *et al.*, (2013) studied on use of bee attractants, Bee-Q and Fruit Boost in the pollination of Niger. Bee visitations to Niger flowers were observed for two weeks and an estimation of seed yield was determined. Results indicate that applications of Bee-Q at 12.5 gm/lit and Fruit boost at 0.75 ml/lit on Niger plots significantly increased the number of bee foragers over control plots. In addition, plots sprayed with these bee attractants significantly enhanced the seed set, seed weight, and germination of Niger.

**Table 5: Effectiveness of different bee attractants to attract the bees in Egyptian clover 3<sup>rd</sup> spray.**

Treatments	Bee visits/m <sup>2</sup> /5 min (3 <sup>rd</sup> spray at 10 days after second spray)				
	1 DBS	1 DAS	3 DAS	5 DAS	Mean
T1- Open Pollination- without treatment	20.07 (4.48)*	21.87 (4.68)	20.20 (4.49)	18.53 (4.30)	20.17 (4.49)
T2- Open Pollination- Sugar Solution @ 10%	22.87 (4.78)	23.67 (4.87)	22.13 (4.71)	21.67 (4.65)	22.58 (4.75)
T3- Open Pollination- Coriander @ 1.25%	21.80 (4.67)	22.07 (4.70)	21.27 (4.61)	20.47 (4.52)	21.40 (4.63)
T4- Open Pollination- Jaggery @ 10%	23.20 (4.82)	24.67 (4.97)	22.67 (4.76)	22.00 (4.69)	23.13 (4.81)
T5- Open Pollination- Cacambe @ 10%	24.73 (4.97)	26.80 (5.18)	24.93 (4.99)	22.80 (4.78)	24.82 (4.98)
T6- Open Pollination- Onion Solution @ 10%	22.27 (4.72)	22.53 (4.75)	21.73 (4.66)	20.33 (4.51)	21.72 (4.66)
T7- Open Pollination- Sugarcane Juice @ 10%	23.80 (4.88)	25.53 (5.05)	22.87 (4.78)	22.20 (4.71)	23.60 (4.86)
T8- Open Pollination- Bee-Q @ 1.25%	24.33 (4.93)	26.67 (5.16)	24.53 (4.95)	22.80 (4.77)	24.58 (4.96)
<b>C.D.**</b>	0.068	0.082	0.063	0.124	0.037

DBS – Day Before Spray; DAS – Day After Spray \*square root transformed values \*\*significant at 5% level of significance

**Figure 4 Graph showing the effectiveness of different bee attractants to attract the bees in Egyptian clover in 3<sup>rd</sup> spray.**



### **Effect of bee pollination on quality and quantity of Egyptian clover.**

The crop sowing practices were initiated during the first fortnight of the month of November, 2020 following all the recommended agronomic practices. The field was kept unsprayed by pesticides throughout the crop season. For evaluating the effect of various combinations of pollination on the yield parameters (quantitative and qualitative), ten treatments were taken for evaluation and were categorised into (OP) Open Pollination (T1 to T8), Caged with bee or (BP) Bee Pollination (T9) and (WIP) Without Insect Pollination or Caged without bees (T10).

### **Effect of different modes of pollination on number of florets per raceme of *T. alexandrinum*:**

Different combinations of mode of pollinations were studied and the analysis of the observed data in table 6 revealed that the insect pollination had a significant effect on the number of florets per raceme. As observed in this experiment, *T. alexandrinum* flower raceme on an average contained 60.27 to 71.47 florets. In mean data highest values were obtained from open pollination plots treated with different combinations of bee attractants, followed by the plot caged with *A. mellifera* colony hives and plants caged without the direct access to any of the pollinating agents. The plot treated with Cacambe 10 percent under open pollination conditions was observed to produce highest mean number of florets *i.e.* 71.47 florets per raceme. This was found at par with the plot treated with Bee-Q 1.25 percent under open pollination conditions producing 70.87 florets per raceme followed by treatment plot treated with Sugarcane juice 10 percent and Jaggery 10 percent producing 68.60 and 67.73 florets per raceme, being statistically same. The next best combinations of pollination were the plots treated with sugar solution 10 percent under open pollination, crop plot caged with bees (or bee pollination), onion solution 10 percent and coriander 1.25 percent under open pollination producing 66.53, 65.60, 64.47 and 62.67 florets per raceme. The two combinations, Open pollination without any treatment and the crop plot caged without bees or any insect (or without insect pollination) were found to be the least effective pollination method in *T. alexandrinum* and recorded lowest mean florets *i.e.*

**Table 6 Effect of bee pollination on quality and quantity of Egyptian clover.**

<b>Treatment Name</b>	<b>No. of Florets/ raceme</b>	<b>No. of Seeds/ raceme</b>	<b>Seed Setting (%)</b>	<b>No. of Seeds/ 50 raceme</b>	<b>1000 Seed wt. (g)</b>
<b>T1- Open Pollination- without treatment</b>	61.00	27.60	42.26	1380.00	2.75
<b>T2- Open Pollination- Sugar Solution @ 10%</b>	66.53	52.73	62.91	2636.67	3.85
<b>T3- Open Pollination- Coriander @ 1.25%</b>	62.67	31.47	45.12	1573.33	3.05
<b>T4- Open Pollination- Jaggery @ 10%</b>	67.73	55.33	64.67	2766.67	4.40
<b>T5- Open Pollination- Cacambe @ 10%</b>	71.47	60.20	66.61	3010.00	4.64
<b>T6- Open Pollination- Onion Solution @ 10%</b>	64.47	36.73	49.02	1836.67	3.41
<b>T7- Open Pollination- Sugarcane Juice @ 10%</b>	68.60	56.27	64.92	2813.33	4.42
<b>T8- Open Pollination- Bee-Q @ 1.25%</b>	70.87	59.27	66.14	2963.33	4.63
<b>T9- Caged with Bees or Bee Pollination (OP)</b>	65.60	49.53	60.34	2476.67	3.60
<b>T10- Caged without bees or Without Insect Pollination (WIP)</b>	60.27	20.93	36.11	1046.67	2.49
<b>C.D.*</b>	<b>0.88</b>	<b>1.20</b>	<b>1.33</b>	<b>60.04</b>	<b>0.12</b>

\*Significant at 5% level of significance.

61.00 and 60.27 florets per raceme respectively. From the foregoing discussion, it was clear that the natural pollinators may play a vital part in clover pollination under both experimental and field conditions.

This is in the general concept that, if seed setting increases then, number of florets will also increase. So, with this general phenomenon, the present findings are supported by Cecen *et al.*, (2007) who reported the highest seed yield of white clover, *Trifolium repens* L. was found in the caged honeybee treatment (46.2 kg/ha) followed by caged bumblebees (37.3kg/ha), open-pollinated (25.7kg/ha) and pollinator excluded (1.37kg/ha). Singh *et al.*, (2012) also reported higher per plot (18 m<sup>2</sup>) seed yield in plots caged with *Apis mellifera* colony (8-frame) as compared to plots caged without insect pollination. Honey bees are reported to be the primary and absolutely essential pollinators of Egyptian clover, visiting it avidly for nectar and pollen and significantly increasing seed yield. Roy *et al.*, (2005) found that for caged Berseem clover where pollinators were excluded, seed setting was reduced by 12 to 99% compared to open pollination across all varieties, even in self-compatible lines.

**Effect of different modes of pollination on number of seeds per raceme of *T. alexandrinum*:**

Different combinations of mode of pollinations were studied and the analysis of the observed data in table 6 revealed that the insect pollination had a significant effect on the number of seeds per raceme. The plot treated with Cacambe 10 percent under open pollination conditions was observed to produce highest mean number of seeds *i.e.* 60.20 seeds per raceme. This was found at par with the plot treated with Bee-Q 1.25 percent under open pollination conditions producing 59.27 seeds per raceme followed by treatment plot treated with Sugarcane juice 10 percent and Jaggery 10 percent producing 56.27 and 55.33 seeds per raceme, being statistically same. The next best combinations of pollination were the plots treated with sugar solution 10 percent under open pollination, crop plot caged with bees (or bee pollination), onion solution 10 percent and coriander 1.25 percent under open pollination producing 52.73, 49.53, 36.73 and 31.47 seeds per raceme. The treatment combination in which the crop was caged without insect pollination was found to be the least effective pollination method in *T. alexandrinum* and recorded

lowest mean *i.e.* 20.93 seeds per raceme. From the foregoing discussion, it was clear that the natural pollinators play a significant role in clover pollination under both experimental and field conditions.

These findings are in line with those of Sattigi *et al.*, 2001a, Guruprasad (2001) found the most seeds/umbel in an open pollinated Niger crop treated with several attractants. Similarly, Viraktamath and Patil (1999) and Patil *et al.*, (2000) observed a larger quantity of seeds when the sesamum crop was treated with Bee-Q and Bee-hera. Choudhary and Kumar (1998) also found that the treated open pollinated Niger crop had a greater percentage of seeds per head (12.90 percent) than the control.

#### **Effect of different modes of pollination on seed setting of *T. alexandrinum*:**

Different combinations of mode of pollinations were studied and the analysis of the observed data in table 6 revealed that the insect pollination had a significant effect on seed setting percent. As observed in this experiment, percent seed setting in *T. alexandrinum* on an average contained 36.11 to 66.61 percent. The plot treated with Cacambe 10 percent under open pollination conditions was observed to produce the highest seed set of 66.61 percent. This was found at par with the plot treated with Bee-Q 1.25 percent, sugarcane juice 10 percent and jaggery 10 percent under open pollination conditions producing 66.14, 64.92 and 64.67 percent seed set being statistically same with each other. The next best combinations of pollination were the plots treated with sugar solution 10 percent under open pollination, crop plot caged with bees (or bee pollination), onion solution 10 percent and coriander 1.25 percent under open pollination producing 62.91, 60.34, 49.02 and 45.12 percent seed set. The crop plot caged without bees or any insect (or without insect pollination) were found to be the least effective pollination method in *T. alexandrinum* and recorded the lowest mean florets *i.e.* 36.11 percent seed set respectively.

The current study clearly demonstrated that employing various bee attractants as a crop treatment to attract diverse pollinators resulted in a substantially greater degree of seed setting. The present studies thus, clearly indicated that employing honey bee as an agent of pollination and open

pollination where honey bees are the major constituent greatly increases seeds but chooses to achieve maximum seed setting to even more intensively or "saturated pollination" .

These findings are amply supported by the findings of Roy *et al.*, (2005) reported that seed setting in 12.3-99.2% was reduced when compared to open pollinated conditions. Malaviya *et al.*, (1999) found that WIP reduced diploid and tetraploid lines by 83.5-84.7% and 60.6-78.4%, respectively, as compared to open pollination. Caging with *A. florea* increased seed set somewhat, but caging with *A. mellifera* resulted in decreased seed set loss (18.7- 37.8 percent ). Singh *et al.*, (2012) found that open pollination (62.46 percent ) and bee pollination with a 4-frame *A. mellifera* colony (61.01 percent ) resulted in the highest seed setting (40.33 percent ) and insect pollination resulted in the lowest (40.33 percent ). Similarly, several studies have found that bee pollination produces more seed than open pollination. Bakheit (1989) discovered the maximum seed setting in bee pollination with *A. mellifera* (51.9%), followed by manual pollination (17.1%), and the lowest under caged circumstances (0.96 percent ). Many researchers, including those included in the current study, have attempted various techniques of pollination (Chowdhury *et al.*, 1966; Narayanan *et al.*, 1961; Singh *et al.*, 2012) and recognised the requirement for honey bees to improve seed production (McGregor, 1976; Free, 1960). However, there is a noticeable lack of effort in using different densities of bee populations, which may be the cause of the decreased seed set observed by these workers. However, these investigations clearly show that saturation pollination provides the most benefits, with the highest (81.4 percent) seed setting observed.

**Effect of different modes of pollination on the (seed yield) number of seeds per 50 raceme of *T. alexandrinum*:**

The quantitative seed production produced by Egyptian clover is the ultimate expression of the symbiotic relationship it had with diverse floral visitors as a consequence of the pollination and fertilization process. As observed in this experiment, *T. alexandrinum* flower raceme on an average contained 1046.67 to 3010.00 seeds per 50 raceme and the observations are listed in table 6. The plot treated with Cacambe 10 percent under open pollination

conditions was observed to produce highest mean number of seeds *i.e.* 3010.00 seeds per 50 raceme. This was found at par with the plot treated with Bee-Q 1.25 percent under open pollination conditions producing 2963.33 seeds per 50 raceme followed by treatment plot treated with Sugarcane juice 10 percent and Jaggery 10 percent producing 2813.33 and 2766.67 seeds per 50 raceme, being statistically same. The next best combinations of pollination were the plots treated with sugar solution 10 percent under open pollination, crop plot caged with bees (or bee pollination), onion solution 10 percent and coriander 1.25 percent under open pollination producing 2636.67, 2476.67, 1836.67 and 1573.33 seeds per 50 raceme. The crop plot caged without bees or any insect (or without insect pollination) were found to be the least effective pollination method in *T. alexandrinum* and recorded lowest mean number of seeds *i.e.* 1046.67 seeds per 50 raceme. The present study thus, clearly indicated the prime importance of bee pollination to boost up and in achieving the higher seed yield in Egyptian clover.

The previous studies in contrast reported the increased seed yields in open pollination conditions (Singh *et al.*, 2012) at 136 g/18m<sup>2</sup> plot which was followed by plots with 4-frame *A. mellifera* colony hive (130.67 g) and least (60.50 g) in plots caged without any insect pollination. The current studies also made it abundantly clear that even after employing natural pollination or open pollination and components of lower and medium honey bee pollination intensities (BP-2F and 4F), there was still enormous scope for further pollinating the florets, as evidenced by additional higher seed yields in saturated pollination (BP-8F) of 25.9% compared to open pollination and 31.0 % o (BP-4F).

Therefore, it may be stated that the intensity of honeybee visits, particularly *Apis mellifera* on niger crop, may be highly efficient in increasing agricultural productivity, in addition to assisting in the production of valuable honey as a source of revenue for farmers. The qualitative parameters of seeds *viz.*, germination ability and oil content also positively improved when visits of pollinators were allowed during blooming phase of niger crop. These results also corroborated the early finding of several workers on different leguminous fodder crops (Rao *et al.*, 1980, Deshmukh *et al.*, 1985, Panda *et al.* 1989, Abrol 1996, Panda *et al.* 1996, Prasad 1998, Kannan 2002).

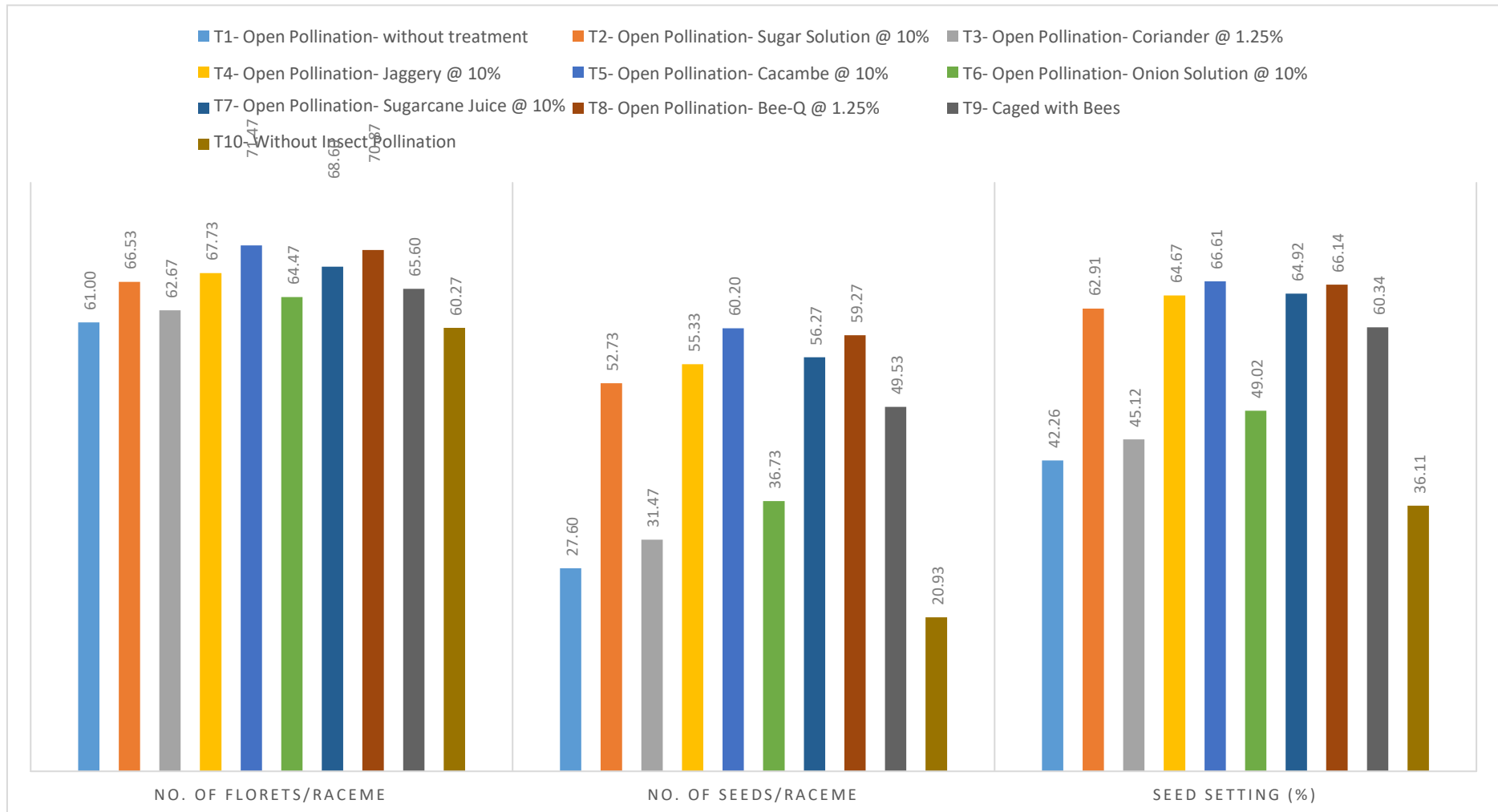
**Effect of different modes of pollination on 1000 seed weight of *T. alexandrinum*:**

Seed weight is a direct indicator of seed health and vigour, and it is used to assess the economic characters (quantitative and qualitative) characteristics of plant. A bolder and heavier seed is the outcome of proper post-pollination and fertilization growth processes, and it is seen to be superior than normal or shrivelled seeds. Especially in a crop like Egyptian clover where seed has no other purpose like food or feed, a bolder seed is an indicator of better germination by providing more food reserve to the germination seedlings. The 1000 seed weight thus is an index of basic seed characteristics. Different combinations of mode of pollinations were studied and the analysis of the observed data in table 6 revealed that the insect pollination had a significant effect on the weight of 1000 seeds. As observed in this experiment, the 1000 seeds weight of *T. alexandrinum* flower raceme ranges from 2.75 to 4.64g. The plot treated with Cacambe 10 percent under open pollination conditions was observed to produce highest mean weight of 1000 seeds *i.e.* 4.64g. This was found at par with the plot treated with Bee-Q 1.25 percent under open pollination conditions producing 4.63g followed by treatment plot treated with Sugarcane juice 10 percent and Jaggery 10 percent producing 4.42 and 4.40g, being statistically same. The next best combinations of pollination were the plots treated with sugar solution 10 percent under open pollination, crop plot caged with bees (or bee pollination), onion solution 10 percent and coriander 1.25 percent under open pollination weights 3.85, 3.60, 3.41 and 3.05g per 1000 seeds respectively. The crop plot caged without bees or any insect (or without insect pollination) was found to produce lightest seed of *T. alexandrinum* and recorded lowest mean weight *i.e.* 2.49g per 1000 seeds. As a consequence, the results, although indicating the necessity for insect visitation, also reveal a small increase in seed weight when varied combinations of pollination are used in comparison to without insect pollination.

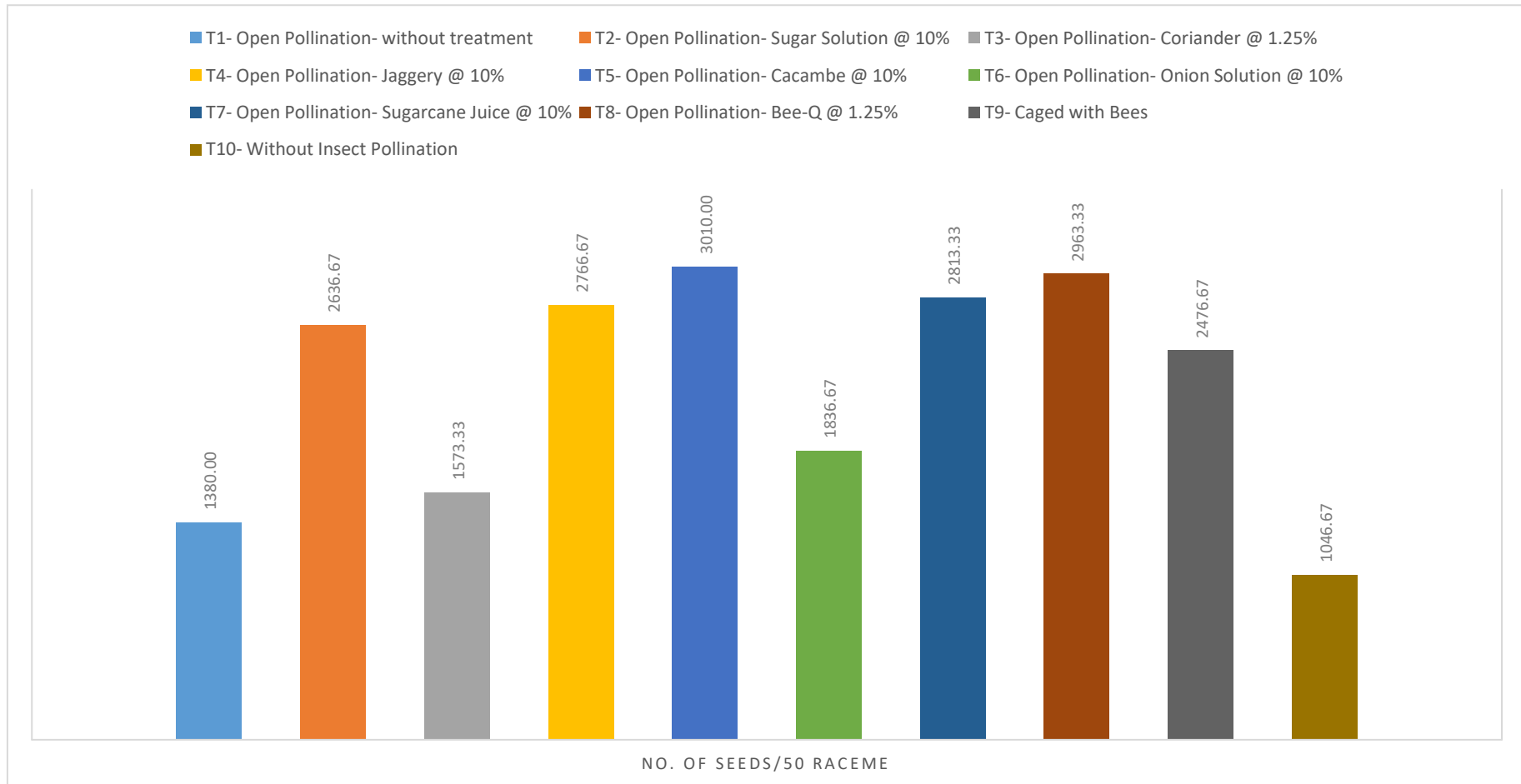
The present results were got supported by Sattigi *et al.* (2001b) and Guruprasad (2001) who worked efficiently on Niger, Viraktamath and Patil (1999) on sesamum who reported the significant increase in various quantitative and qualitative characters line number of florets, number of seeds, seed set, seed yield, 1000 seeds weight, germination and seed vigour of

different crops in different treated open pollination situation, bee pollination and without insect pollination. These results also justifies the findings of Free (1993) who also reported higher 1000 seeds weight in bee pollinated plots (13.8 g) in comparison with to only 0.2 g in without insect pollination but was significantly lower in open pollination conditions(6.1 g).

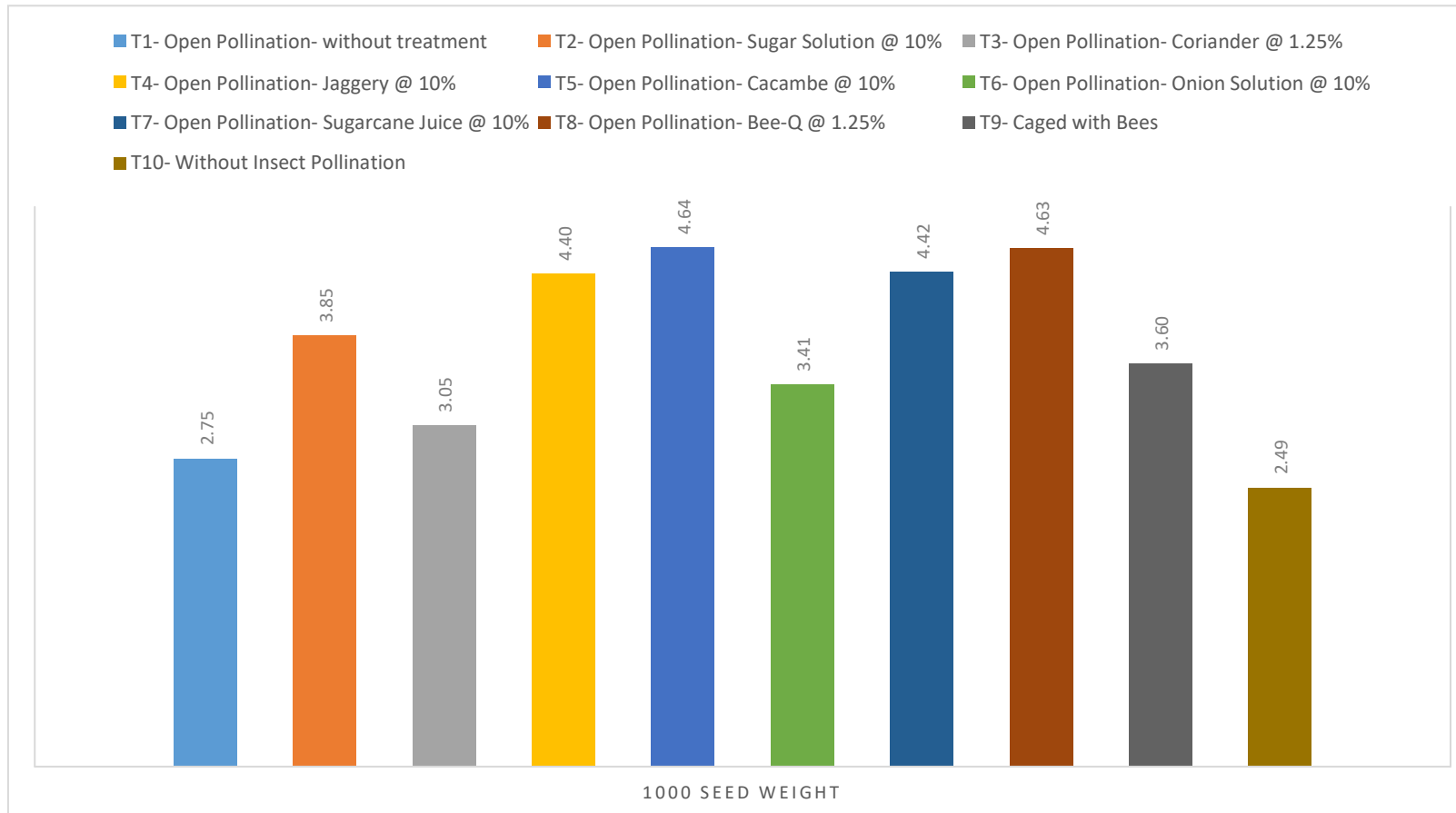
**Figure 5: Graph showing the effect of bee pollination on number of florets and seeds per raceme and seed setting percent.**



**Figure 6: Graph showing the effect of bee pollination on number of seeds per 50 raceme.**



**Figure 7: Graph showing the effect of bee pollination on 1000 seed-weight.**



## CHAPTER V

# SUMMARY, CONCLUSION AND SUGGESTIONS FOR FURTHER WORK

The present investigations entitled, “**To study the pollination behaviour and effect of honey bee pollination on quantitative and qualitative parameters of Egyptian Clover *Trifolium alexandrinum* L.**” was carried in the assigned experimental field of Egyptian clover and the apiary at RVSKVV- Krishi Vigyan Kendra, Morena, Madhya Pradesh, during 2020-21. In this chapter the salient findings of the investigations are summarized and meaningful conclusions have been made as under.

### **To study the pollinator fauna of Egyptian clover.**

Studies on the visit of pollinators' fauna in the field of Egyptian clover revealed that about seventeen species of insects were observed and are listed to give a general picture of the arthropod specimens that have been identified. This list includes all the insects that have visited the field during the observation whether they are harmful or beneficial to the crop. However, listed arthropods represent 18 genera of Arthropods belong to 13 families and 7 orders. The identified insect species that are listed in this table were classified into Phytophagous, Predator, and Nectarivore according to their behaviour.

Order Hymenoptera was observed to be the most dominant and includes the most active pollinators' with 44.44 percent dominance followed by Lepidopterans, Dipterans, Coleopterans, Hemipterans, Neuropterans, and Odonata which were found at 16.66, 11.11, 11.11, 5.55, 5.55, and 5.55 percent dominance, respectively (Fig. 1). Bee pollinators visiting during flowering time includes *Apis dorsata*, *Apis mellifera*, *Apis cerana indica* and *Apis florea*. The study also shows that *Apis dorsata* mainly targets the upper region of crop canopy, while *Apis mellifera*, *Apis cerana indica* and *Apis florea* forages in the flowers of lower region of crop canopy. Thus, it shows that *A. dorsata* is more effective and predominant pollinators with good frequency in berseem followed by *Apis mellifera* and *Apis florea*.

The study further reveals that the pollinators starts their activity from 0800 hours and remains active until 1700 hours. Most of the pollinators'

remains active in the morning hours. The peak activity of pollinators was observed approximately at 1000 hours followed by drop in the activity during noon time. Even though bee activity again increases in the afternoon near about 1200 hours and those bees are assumed to be nectar collectors rather than pollen gatherers. Later the activity of pollinators in the evening hours was observed to be less.

### **To study the effectiveness of different bee attractants to attract the bees in Egyptian clover.**

Influence of attractants on visitation of *A. mellifera* was studied and the observations on bee visitation to Egyptian clover crop sprayed with different bee attractants at different flowering stages are described and briefly discussed on the basis of number of sprays and flowering stage.

#### **First spray**

In order to achieve the proper and efficient pollination and to improve the qualitative and quantitative characteristics of the Egyptian clover, the efficacy of several bee attractant sprays in the Egyptian clover crops was evaluated during the flowering stage with 1<sup>st</sup> spray at 10 percent flowering stage.

A day before spray the treatments applied to the crop were found to be non-significant. During this bee activity ranges from 18.40 to 18.73 bees/m<sup>2</sup>/5min.

On one day after spray of the treatment the effectiveness of Cacambe 10 percent was found to be significantly most effective and superior treatment in attracting higher number of bees *i.e.*, 35.60 bees/m<sup>2</sup>/5min and it was found at par with 1.25 percent Bee-Q attracting 34.53 bees/m<sup>2</sup>/5min. The least effective treatment was observed in the plot under open pollination without any treatment (21.87 bees/m<sup>2</sup>/5min).

On 3<sup>rd</sup> day after the first spray, the same trend of visit of bees to the treated plots was observed. Cacambe 10 percent (34.20 bees/m<sup>2</sup>/5min) was significantly superior in attracting greater number of bees followed by 33.33 bees/m<sup>2</sup>/5min in the plot treated with Bee-Q 1.25 percent. The lowest number of bees were observed in treatments with open pollination without any treatment (20.07 bees/m<sup>2</sup>/5min).

On 5th day after first spray, treatment Cacambe 10 percent and Bee-Q 1.25 percent were found to be effective in attracting highest number of bees, *i.e.* 28.47 and 28.00 bees/m<sup>2</sup>/5min respectively, being the superior treatment over other treatments showing the statistically same effects. However, the least effective treatment was untreated control, thus attracting 22.20 bees/m<sup>2</sup>/5min.

On an average Cacambe 10 percent showed relatively highest bee activities with 29.25 bees/m<sup>2</sup>/5min which was found to be having statistically same effects with the plot treated with Bee-Q 1.25 percent showing 28.63 bees/m<sup>2</sup>/5min. The least average bee activities were observed in untreated control plot (20.63 bees/m<sup>2</sup>/5min).

### **Second spray**

The effectiveness of several bee attractants sprays on Egyptian clover crop during flowering phase at 2<sup>nd</sup> spray after 10 days from the 1<sup>st</sup> spray to attract *A. mellifera* for proper pollination and to enhance the qualitative and quantitative characters of Egyptian clover was evaluated.

A day before second spray, bee activity of *A. mellifera* was observed ranging from 20.93 to 25.53 bees/m<sup>2</sup>/5min. The effectiveness of the treatments Cacambe 10 percent and Bee-Q 1.25 percent applied in the crop were found at par (25.53 and 25.07 bees/m<sup>2</sup>/5min as the flowering was at near its peak. The least influencing treatment observed was untreated control (20.93 bees/m<sup>2</sup>/5min).

One day after second spraying, the treatment with Cacambe 10 percent and Bee-Q 1.25 percent were found significantly superior and statistically same in attracting a greater number of bees (37.60 and 36.53 bees/m<sup>2</sup>/5min. Without any treatment spray or open pollination was recorded to attract the lowest number of bees (24.07 bees/m<sup>2</sup>/5min).

Among various treatments applied to plots, spraying of Cacambe 10 percent was found to be superior to attract 35.60 bees/m<sup>2</sup>/5min compared to other treatments on the 3<sup>rd</sup> day after second spray and was found at par with Bee-Q 1.25 percent (35.20 bees/m<sup>2</sup>/5min). However, the treatment with open pollination, which recorded as 21.87 bees/m<sup>2</sup>/5min found to be lowest bee attracting treatment.

On 5th day after second spray Cacambe 10 percent, Bee-Q 1.25 percent, Sugarcane juice 10 percent and Jaggery 10 percent attracted maximum number of bees ranging from 27.67 to 28.87 bees/m<sup>2</sup>/5min, found significantly superior over the other treatments and were on par with each other. Untreated control (21.53 bees/m<sup>2</sup>/5min) which was ineffective in attracting more bees.

Cacambe 10 percent showed relatively highest bee activities with 31.90 bees/m<sup>2</sup>/5min on average throughout the period of observation on second spray, which was found to have statistically same effects with the plot treated with Bee-Q 1.25 percent showing 31.30 bees/m<sup>2</sup>/5min. The untreated control plot had the lowest average bee activity (22.10 bees/m<sup>2</sup>/5min).

### **Third spray**

The effectiveness of several bee attractants sprays on Egyptian clover crop during flowering phase at 3<sup>rd</sup> spray after 10 days from the 2<sup>nd</sup> spray to attract *A. mellifera* for proper pollination and to increase the qualitative and quantitative characters of Egyptian clover was evaluated, and the results are listed in Table 5.

A day before third spray, bee activity of *A. mellifera* was observed ranging from 20.07 to 24.73 bees/m<sup>2</sup>/5min. Cacambe 10 percent was found to attract 24.73 bees/m<sup>2</sup>/5min as the flowering stage is going to complete. The least influencing treatment observed was untreated control (20.07 bees/m<sup>2</sup>/5min).

One day after third spraying, the treatment with Cacambe 10 percent and Bee-Q 1.25 percent were found significantly superior and statistically same in attracting a greater number of bees (26.80 and 26.67 bees/m<sup>2</sup>/5min respectively). Open pollination (without any treatment spray) observing the lowest number of bees (21.87 bees/m<sup>2</sup>/5min).

Among different treatments, spraying of Cacambe 10 percent was found to be superior compared to other treatments (24.93 bees/m<sup>2</sup>/5min) on the 3<sup>rd</sup> day after third spray and the treatment with open pollination, which was recorded as 20.20 bees/m<sup>2</sup>/5min found to be lowest bee attracting treatment.

On 5th day after third spray Cacambe 10 percent, Bee-Q 1.25 percent, Sugarcane juice 10 percent, Jaggery 10 percent and sugar solution 10 percent

attracted statistically same number of bees ranging from 21.67 to 22.80 bees/m<sup>2</sup>/5min, found significantly over the other treatments. Control (18.53 bees/m<sup>2</sup>/5min) was ineffective in attracting more bees.

Cacambe 10 percent showed relatively highest bee activities with 24.82 bees/m<sup>2</sup>/5min on average throughout the period of observation on third spray, which was found to have statistically same effects with the plot treated with Bee-Q 1.25 percent showing 24.58 bees/m<sup>2</sup>/5min. The untreated control plot had the lowest average bee activity (20.17 bees/m<sup>2</sup>/5min).

### **Effect of bee pollination on quality and quantity of Egyptian clover.**

The crop sowing practices were initiated during the first fortnight of the month of November, 2020 following all the recommended agronomic practices. The field was kept unsprayed by pesticides throughout the crop season. To assess the impact of various pollination combinations on quantitative (seed yield) and qualitative yield parameters, a total combinations of ten treatments were taken for evaluation.

### **Effect of different modes of pollination on number of florets per raceme of *T. alexandrinum*:**

Different combinations of mode of pollinations were studied and the analysis of the observed data in table 6 revealed that the insect pollination had a significant effect on the number of florets per raceme. As observed in this experiment, *T. alexandrinum* flower raceme on an average contained 60.27 to 71.47 florets. In mean data highest values were obtained from open pollination plots treated with various combinations of bee attractants, followed by the plots with bee pollination and plants caged without insect pollinators. The plot treated with Cacambe 10 percent under open pollination conditions was observed to produce highest mean number of florets *i.e.* 71.47 florets per raceme. This was found at par with the plot treated with Bee-Q 1.25 percent under open pollination conditions producing 70.87 florets per raceme followed by treatment plot treated. Plot caged without bees or any insect (or without insect pollination) were found to be the least effective pollination method in *T. alexandrinum* and recorded lowest mean florets *i.e.* 61.00 and 60.27 florets per raceme respectively.

**Effect of different modes of pollination on number of seeds per raceme of *T. alexandrinum*:**

Different combinations of mode of pollinations were studied and the analysis of the observed data in table 6 revealed that the insect pollination had a significant effect on the number of florets per raceme. The plot treated with Cacambe 10 percent under open pollination conditions was observed to produce highest mean number of seeds *i.e.* 60.20 seeds per raceme. This was found at par with the plot treated with Bee-Q 1.25 percent under open pollination conditions producing 59.27 seeds per raceme. The treatment without insect pollination was found to be the least effective pollination method in *T. alexandrinum* and recorded lowest mean *i.e.* 20.93 seeds per raceme.

**Effect of different modes of pollination on seed setting of *T. alexandrinum*:**

Different combinations of mode of pollinations were studied and the analysis of the observed data in table 6 revealed that the insect pollination had a significant effect on seed setting percent. As observed in this experiment, percent seed setting in *T. alexandrinum* on an average contained 36.11 to 66.61 percent. The plot treated with Cacambe 10 percent under open pollination conditions was observed to produced highest seed set of 66.61 percent. This was found at par with the plot treated with Bee-Q 1.25 percent producing 66.14 percent seed set. The crop plot caged without bees or any insect (or without insect pollination) were found to be the least effective pollination method in *T. alexandrinum* and recorded lowest mean florets *i.e.* 36.11 percent seed set respectively.

**Effect of different modes of pollination on number of seeds per 50 raceme (seed yield) of *T. alexandrinum*:**

As observed in this experiment, *T. alexandrinum* flower raceme on an average contained 1046.67 to 3010.00 seeds per 50 raceme and the observations are listed in table 6. The plot treated with Cacambe 10 percent under open pollination conditions was observed to produce highest mean number of seeds *i.e.* 3010.00 seeds per 50 raceme. This was found at par with the plot treated with Bee-Q 1.25 percent under open pollination conditions producing 2963.33 seeds per 50 raceme. The crop plot caged without bees or

any insect (or without insect pollination) were found to be the least effective pollination method in *T. alexandrinum* and recorded lowest mean number of seeds *i.e.* 1046.67 seeds per 50 raceme.

**Effect of different modes of pollination on 1000 seed weight of *T. alexandrinum*:**

Seed weight is a direct indicator of seed health and vigour, and it is used to assess the economic characters (quantitative and qualitative) characteristics of plant. Different combinations of mode of pollinations were studied and the analysis of the observed data in table 6 revealed that the insect pollination had a significant effect on the weight of 1000 seeds. As observed in this experiment, the 1000 seeds weight of *T. alexandrinum* flower raceme ranges from 2.75 to 4.64g. The plot treated with Cacambe 10 percent under open pollination conditions was observed to produce highest mean weight of 1000 seeds *i.e.* 4.64g. This was found at par with the plot treated with Bee-Q 1.25 percent under open pollination conditions producing 4.63g. The crop plot caged without bees or any insect (or without insect pollination) was found to produce lightest seed of *T. alexandrinum* and recorded lowest mean weight *i.e.* 2.49g per 1000 seeds.

## CONCLUSION

During the study period, the key results arrived were of the significant importance to farmers who are involved in the production of Egyptian clover for the purpose of forage cut or for the purpose of seed production. These key results are discussed here under:

- Egyptian clover is more profitable when the best pollination combinations were employed in order to increase the green fodder yield as well as seed yield.
- During the whole investigation, *Apis dorsata* mainly targets the upper region of crop canopy and spent lesser time per flower and visited more number of flowers per minute, while *Apis mellifera*, *Apis cerana indica* and *Apis florea* forages in the flowers of lower region of crop canopy and spends more time on individual flowers., it shows that *A. dorsata* is more effective and predominant pollinators with good frequency in berseem followed by *Apis mellifera*, *Apis cerana indica* and *Apis florea*.
- Observations on effectiveness of bee attractants revealed that cacambe 10 percent was found to be the superior bee attractant followed by Bee-Q 1.25 percent, sugarcane juice 10 percent, Jaggery solution 10 percent, Onion solution 10 percent and Coriander 1.25 percent.
- Different pollination combinations incorporated in the study revealed that the experimental plot treated with cacambe 10 percent results in the superior qualitative and quantitative characters as compared to the other treatments. This treatment produced highest number of florets per raceme and number of seeds per raceme. Followed by these characters, higher seed setting percent results in increased seed yield, *i.e.* number of seeds per 50 raceme and 1000 seeds weight.
- Use of honeybee attractants is very important from this point of view. Honeybees mainly communicate with each other by means of vision, taste, touch and smell. This results in successful pollination and increased overall yield, necessary to meet up the demand of the increasing livestock population as well as quality resulting a better feed supplement.

## **SUGGESTIONS FOR FURTHER WORK:**

During the whole study, there are some areas of ample scope for further studies related to crop pollination ecology in the region. Some of the points are mentioned below:-

1. The present studies are conducted in fodder crop, but further studies can also be done in food crops, vegetables and fruits so as to increase the yield quantitatively as well as qualitatively to meet up the future needs.
2. The duration of the study may be extended up to the whole year for annual crops, fruits, etc.
3. Studies on the efficiency of the bee attractants on different concentrations and attractants other than these employed in the present study can be done so as to discover and explore more alternatives for farmers.
4. Other effective methods to increase the seed production as well as the green fodder yield of the Egyptian clover with the help of honey bees needs to develop.
5. There should be effective utilization of attractants to attract honey bees.
6. There are ample opportunities for such studies to be conducted on other bee species, for example, Stingless bee (*Tetragonula iridipennis*), etc.

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## APPENDIX I

Analysis of Variance (ANOVA) for effectiveness of different bee attractants to attract the bees in Egyptian clover in 1<sup>st</sup> spray during rabi 2020. (Table 3)

<b>1 Day Before Spray (DBS)</b>		
<b>Source of variation</b>	<b>Degrees of freedom</b>	<b>Mean sum of squares</b>
<b>Replications</b>	2	0.001
<b>Treatments</b>	7	0.001
<b>Error</b>	14	0.000
<b>Total</b>	23	-

<b>1 Day After Spray (DAS)</b>		
<b>Source of variation</b>	<b>Degrees of freedom</b>	<b>Mean sum of squares</b>
<b>Replications</b>	2	0.011
<b>Treatments</b>	7	0.605
<b>Error</b>	14	0.003
<b>Total</b>	23	-

<b>3 Day Before Spray (DBS)</b>		
<b>Source of variation</b>	<b>Degrees of freedom</b>	<b>Mean sum of squares</b>
<b>Replications</b>	2	0.000
<b>Treatments</b>	7	0.740
<b>Error</b>	14	0.001
<b>Total</b>	23	-

<b>5 Day Before Spray (DBS)</b>		
<b>Source of variation</b>	<b>Degrees of freedom</b>	<b>Mean sum of squares</b>
<b>Replications</b>	2	0.029
<b>Treatments</b>	7	0.222
<b>Error</b>	14	0.035
<b>Total</b>	23	-

<b>Total Average of 1<sup>st</sup> Spray</b>		
<b>Source of variation</b>	<b>Degrees of freedom</b>	<b>Mean sum of squares</b>
<b>Replications</b>	2	0.001
<b>Treatments</b>	7	0.304
<b>Error</b>	14	0.003
<b>Total</b>	23	-

## APPENDIX II

Analysis of Variance (ANOVA) for effectiveness of different bee attractants to attract the bees in Egyptian clover in 2<sup>nd</sup> spray during rabi 2020. (Table 4)

<b>1 Day Before Spray (DBS)</b>		
<b>Source of variation</b>	<b>Degrees of freedom</b>	<b>Mean sum of squares</b>
<b>Replications</b>	2	0.001
<b>Treatments</b>	7	0.096
<b>Error</b>	14	0.001
<b>Total</b>	23	-

<b>1 Day After Spray (DAS)</b>		
<b>Source of variation</b>	<b>Degrees of freedom</b>	<b>Mean sum of squares</b>
<b>Replications</b>	2	0.009
<b>Treatments</b>	7	0.550
<b>Error</b>	14	0.003
<b>Total</b>	23	-

<b>3 Day Before Spray (DBS)</b>		
<b>Source of variation</b>	<b>Degrees of freedom</b>	<b>Mean sum of squares</b>
<b>Replications</b>	2	0.000
<b>Treatments</b>	7	0.688
<b>Error</b>	14	0.002
<b>Total</b>	23	-

<b>5 Day Before Spray (DBS)</b>		
<b>Source of variation</b>	<b>Degrees of freedom</b>	<b>Mean sum of squares</b>
<b>Replications</b>	2	0.018
<b>Treatments</b>	7	0.253
<b>Error</b>	14	0.022
<b>Total</b>	23	-

<b>Total Average of 1<sup>st</sup> Spray</b>		
<b>Source of variation</b>	<b>Degrees of freedom</b>	<b>Mean sum of squares</b>
<b>Replications</b>	2	0.000
<b>Treatments</b>	7	0.362
<b>Error</b>	14	0.001
<b>Total</b>	23	-

### APPENDIX III

Analysis of Variance (ANOVA) for effectiveness of different bee attractants to attract the bees in Egyptian clover in 3<sup>rd</sup> spray during rabi 2020. (Table 5)

<b>1 Day Before Spray (DBS)</b>		
<b>Source of variation</b>	<b>Degrees of freedom</b>	<b>Mean sum of squares</b>
<b>Replications</b>	2	0.002
<b>Treatments</b>	7	0.076
<b>Error</b>	14	0.001
<b>Total</b>	23	-

<b>1 Day After Spray (DAS)</b>		
<b>Source of variation</b>	<b>Degrees of freedom</b>	<b>Mean sum of squares</b>
<b>Replications</b>	2	0.000
<b>Treatments</b>	7	0.123
<b>Error</b>	14	0.002
<b>Total</b>	23	-

<b>3 Day Before Spray (DBS)</b>		
<b>Source of variation</b>	<b>Degrees of freedom</b>	<b>Mean sum of squares</b>
<b>Replications</b>	2	0.002
<b>Treatments</b>	7	0.084
<b>Error</b>	14	0.001
<b>Total</b>	23	-

<b>5 Day Before Spray (DBS)</b>		
<b>Source of variation</b>	<b>Degrees of freedom</b>	<b>Mean sum of squares</b>
<b>Replications</b>	2	0.035
<b>Treatments</b>	7	0.079
<b>Error</b>	14	0.005
<b>Total</b>	23	-

<b>Total Average of 1<sup>st</sup> Spray</b>		
<b>Source of variation</b>	<b>Degrees of freedom</b>	<b>Mean sum of squares</b>
<b>Replications</b>	2	0.002
<b>Treatments</b>	7	0.086
<b>Error</b>	14	0.000
<b>Total</b>	23	-

## APPENDIX IV

Analysis of Variance (ANOVA) for effectiveness of different bee attractants to attract the bees in Egyptian clover in 3<sup>rd</sup> spray during rabi 2020. (Table 6)

<b>No. of florets per raceme</b>		
<b>Source of variation</b>	<b>Degrees of freedom</b>	<b>Mean sum of squares</b>
<b>Replications</b>	2	1.225
<b>Treatments</b>	9	45.003
<b>Error</b>	18	0.262
<b>Total</b>	29	-

<b>No. of seeds per raceme</b>		
<b>Source of variation</b>	<b>Degrees of freedom</b>	<b>Mean sum of squares</b>
<b>Replications</b>	2	1.617
<b>Treatments</b>	9	627.367
<b>Error</b>	18	0.490
<b>Total</b>	29	-

<b>Seed Setting percent</b>		
<b>Source of variation</b>	<b>Degrees of freedom</b>	<b>Mean sum of squares</b>
<b>Replications</b>	2	0.222
<b>Treatments</b>	9	395.896
<b>Error</b>	18	0.603
<b>Total</b>	29	-

<b>No. of seeds per 50 raceme (Seed yield)</b>		
<b>Source of variation</b>	<b>Degrees of freedom</b>	<b>Mean sum of squares</b>
<b>Replications</b>	2	4043.333
<b>Treatments</b>	9	1568418.148
<b>Error</b>	18	1224.815
<b>Total</b>	29	-

<b>1000 seed weight</b>		
<b>Source of variation</b>	<b>Degrees of freedom</b>	<b>Mean sum of squares</b>
<b>Replications</b>	2	0.002
<b>Treatments</b>	9	1.884
<b>Error</b>	18	0.004
<b>Total</b>	29	-

## VITA

The author of this thesis is Mr. Kanhaiya Lal Kumawat s/o Badrilal Kumawat born on 05<sup>th</sup> May 1996 at Daloda, Mandsaur (Madhya Pradesh). He passed his high school education in 2012 from School of Excellence, Mandsaur, Madhya Pradesh (MP Board) with 90%. He passed his senior secondary examination in 2014 from School of Excellence, Mandsaur, Madhya Pradesh (MP Board) with 82.6%. He completed his graduation in B.Sc. Agriculture from College of Agriculture, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, Madhya Pradesh in the year 2019 and achieved OGPA of 7.57 / 10. After completion of graduation, he was admitted in College of Agriculture, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, Madhya Pradesh for M.Sc. (Ag.) Entomology in the year 2019. He did research on the topic "To study the pollination behaviour and effect of honey bee pollination on quantitative and qualitative parameters of Egyptian Clover *Trifolium alexandrinum* L."

Date:

Place: Gwalior

Kanhaiya Lal Kumawat