Incidence,Biology and Management of Saffron Bulb Mite *Rhizoglyphusrobini*(Claparede)on Saffron Corms in Kashmir

Abstract

Fieldstudywas conducted at different saffron growing areas of Kashmir and laboratory study at Department of Entomology, Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Shalimar(India) to study the incidence biology and management of Saffron bulb mite *Rhizoglyphusrobini* (Sarcoptiformes: Acaridae) on saffron corms in Kashmir.The mean percent infestation of saffron bulb mite from all the locations revealed highest and lowest percent mite infestation of 25.83and 12.50recorded fromDhussu and Saffron research station respectively.Passing through five active stages viz., Egg, Larva, Protonymph, Tritonymph and adult completed the life cycle of *Rhizoglyphus robini*. The heteromorphic deutonymph or hypopal stage is sometimes producedfor phoresiswhen population is overcrowded, but this stage was not observed during the study. The results revealed that the females laid their eggs on the corms. The average incubation period of egg was observed to be of 6.16 ±0.78 days while as other developmental periods ~~i.e.~~i.e.,Larva, Protonymph, and Tritonymph were of 5.22 ±0.68, 5.76 ±0.52, and 7.00 ±0.91 daysat 25±5^{0}C respectively. The longevity of adult male and female were27.9 ±0.44 and22.9 ±0.69days,respectively. The efficacy of different doses of acaricides against Saffron bulb mite revealed that drenching the soil with chlorpyrifos 20 EC + foliar spray with cyenopyrafen30 SC showed best results with 4.23 percent leaves with stunted growth and chlorosis, 97.34 percent flower emergence and 2.23 percent bulb rot. Themesostigmatic soil-dwelling predatory mite *Hypoaspisaculeifer* was found in association with the bulb mite, *Rhizoglyphusrobini*.

Keywords: Biology, *Rhizoglyphusrobini*, Saffron, *Hypoaspisaculeifer*, Management.

Introduction

Mites are the smallest and less studied among the arthropods, many are predaceous on small invertebrates in diverse ecological niches. Some may be phytophagous and cause considerable damage to crops and stored food products, while others are animal parasites capable of transmitting a variety of pathogens.*Rhizoglyphus*is a genus of the family Acaridae,withworldwide distribution and~~. It~~ its speciesare associated with plants or plant products (Manson 1972), mostly occurring with various types of bulbs in the field,greenhouse, andstorage. Fumouze and Robin (1868) described the first species of the genus *Rhizoglyphus*with the name *Tyroglyphusechinopus*.Little information is available on the biology of *Rhizoglyphus* spp. except for the brief reports by Hodson (1928) and Garman (1937). Bulb mites in the

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Genus *Rhizoglyphus* are mostly unseen creatures that spend most of their lives in the soil. They are commonly associated with plants with bulbs, corms and tubers. *Rhizoglyphusrobini*(Claparede), is the most important member of this genus, and is known to cause damage to a variety of crops (e.g. Onions, Garlic, Tulip, Dhalia, Gladiolus, Lily and Saffron)in greenhouses and in the field around the world(Diaz et al. 2000).It is cosmopolitan and one of the most important pests of saffron in field as well as in storage, and. I it is found in all saffron growing belts of the world. The mite generally occurs in clusters inhabiting damaged areas of saffron corms. Bulb mites can survive on decaying crops in the field until it is completely decomposed.In Kashmir, this pest is reported from major saffron growing areas and goes unnoticed due to infestation of corms under the soil. They attack the corms through wounds and from the diseased areas. The infestation is more severe in areas which lack proper sanitation. This pest is active throughout the year in Kashmir but the maximum number is found during spring and fall when optimum conditions exist for growth and reproduction. Keeping in view the economic importance of this pest the present study was formulated with the aim of developing management practices based on the biology.

Material and methods

The experiment was carried out in the Acarology laboratory, Division of Entomology, SKUAST (K) Shalimar during years 2019, 2020 and 2021on the biology and management of saffron bulb mite *R. robini*.

Incidence of *Rhizoglyphus robini*

Saffron corms that were 120 in number were collected randomly from eachlocation viz., Konibal, Dhussu, Khonmoh, Pampore, Chandhoreand Saffron Research Station. Individual samples were packed in a sterile plastic container with their mouth sealed with muslin cloth to prevent moisture formation and were transferred to the laboratory without much disturbance under ambient condition. The samples were observed under binocular microscope (Olympus CX20i). The incidence of bulb mite*R. robini*and predatory mite *Hypoaspisaculeifer* (Canestrini) Parasitiformes, Dermanyssidaewas worked out from the collected corms by isolating the mites from them with soft brush. The percentage of infestation wasobtained by the given formula.

No of infested corms / Total number of corms X 100

The population count of bulb and predatory mite was taken on four sides of the saffron corms with 1 cm^2 quadrant on each side.

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Biology of saffron bulb mite

Collecting and rearing of*Rhizoglyphus robini*

The biology of mites was studied under laboratory conditions from August to November during the years 2019, 2020 and 2021.Infested corms obtained from different locations were examined under a binocular microscope (Olympus CX20i). Adult Gravid females were identified under a stereo binocular microscope transferred and maintained on fresh corms.Gravid females were individually placed overnight onfresh corms with the help of camel brush, and the corms were regularly monitored for laying of eggs. Males died shortly after mating as was prominent from their colour change. Deposited eggs were transferred on the next day tonew corms.

Biological culture

Thirtyeggs of *R. robini*wasused to start a biological laboratoryculture on fresh water-soakedcorms inPetri dish. For the biology study, each egg was placed on a new saffron corm, which was kept in a Petri dish (10 cm in diameter) covered with lid at room temperature of 25 ± 5^0Cin the laboratory. A total of 30 Petri dishes were required. Water was added to cotton beds to keep the corms moist.After hatching, the nymphs of the mites were transferred to fresh saffron cormsthat had their outer covering removed so that nymphs could pierce them easily. Every day the nymphs were measured with respect the length, and any change in length was considered as moulting and beginning of the next instar.The identification of different stages was done with the help of ocular micrometer fitted to the binocular. The ocular micrometer was calibrated with the stage micrometer at 20X.The culture was changed every week to prevent any pathogenic growth and to maintain sanitation.

Management

To develop a management strategy for bulb mite under field conditions, six treatment modules were tested for their efficacy. The trail was laid at the experimental research farm (34^0 8'48 N, 74^0 49' 52 E) of the Division of Entomology, Sher-e-Kashmir University of Agricultural Sciences and Technology Shalimar Srinagar during the autumn of 2019, 2020 and 2021. Saffron crop was raised by following the recommended agronomic practices in a Randomized Block Design (RBD) fashion. The details of the insecticides/acaricides are given in table 1. Each treatment was replicated thrice.The bulbs were sown on raised beds. The spacing between bulb to bulb and row to row was kept as 15 and 30 cm respectively.

Table 1. Classification of insecticides/acaricides used in the present study.

S.No	Insecticide/ acaricide group	IRAC MOA* class	Active ingredient	Mode of action/target site
1.	Neonicotinoids	4A	Imidacloprid	Nicotinic acetylcholine receptor (nAChR) agonist
2.	Organophosphates	1B	Chlorpyrifos	Acetylcholinesterase (inhibitors)
3.	Beta-ketonitrile derivative	25A	Cyenopyrafen	Mitochondrial complex II electron transport inhibitor
4.	Propargite	12C	Propargite	Inhibitor of mitochondrial ATP synthase

*IRAC MOA: Insecticide Resistance Action Committee (IRAC 2021).

Three plants were selected for one replication of each treatment and each treatment was replicated thrice. The method of application of the different chemicals in the various treatment modules is presented in table 4. The formulations of the various chemicals were diluted with water at desired dose and applied with knap sack sprayer. The control plants were drenched, soaked and sprayed in the same way as in the treated ones but with water only.The foliar spray was done at pre flower emergence stage.

Results

Seasonal Incidence

The observations on the incidence of saffron bulb mite and the predatory mite *H. aculeifer*were recorded from 29th standard meteorological week (SMW) (3rd week of July) to 34th standard week (4th week of August) at various locations during 2019, 2020and 2021. The highest population of predatory mite H. aculeifer was found in 33rd and the lowest in the 29th SMW. Perusal of the data in table 2 and Figure 1, revealed that 29 corms were infested with bulb mitecollected from Konibalduring SMW of 33 and the mean per cent infestation was recorded to be 24.16.From Dhussu area,31 corms were infested and mean

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per cent infestationwas recorded as 25.83 during SMW of 30. From Khonmohlocation,21 were found infested and the mean percentage of mite infestation was found to be 17.50 during SMW of 31. From Pampore location,23 corms were found infested with mean per cent infestation of 19.16 in the SMW of 29. From Chandhoralocation,27 were infested with mean percentage of 22.50 in the SMW of 34.The lowest mean per cent infestation of bulb (12.50) and predatory mite (3.22) were observed from Saffron Research Stationduring the SMW of 29.

Table 2. Incidence of Saffron bulb mite *Rhizoglyphus robini* and predatory mite *Hypoaspisaculeifer* in saffron growing areas of Kashmir during the year 2019-21.

Month/SMW	Place of collection	Coordinates	No of corms collected	No of corms infested	*Mean no. of eggs observed	*Mean no. of nymphs observed	*Mean no. of adult observed	Per cent mite infestation	*Mean no. of predatory mite *Hypoaspisaculeifer* observed
August/33	Konibal	34.02^0N74.93^0E	120	29	10.31	21.76	10.78	24.16	8.48
July/30	Dhussu	33.92^0N74.95^0E	120	31	14.54	16.25	11.23	25.83	6.78
August/31	Khonmoh	34.89^0N76.49^0E	120	21	12.87	18.43	7.89	17.50	7.87
July/29	Pampore	34.00^0N74.92^0E	120	23	11.95	15.21	10.89	19.16	6.99
August/34	Chandhore	32.56^0N96.67^0E	120	27	8.32	14.02	7.80	22.50	5.43
July/29	Saffron Research Station	34.02^0N74.93^0E	120	15	6.83	9.04	6.26	12.50	3.22

*Observation taken on 1 cm^2 quadrant on each side.
SMW=standard meteorological week.

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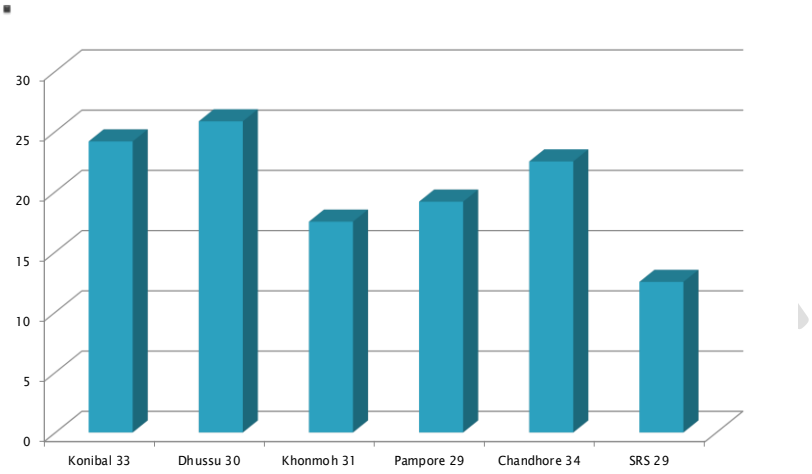

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Figure~~:~~ 1. Incidence of saffron bulb mite at different locations.

Biology of Saffron bulb mite

The different stages in the life cycle of saffron bulb mite studied were egg, larva, protonymph, tritonymph and adult. The duration in days of different life stages of bulb mite *R. robini* is shown in table 3 and diagrammatically represented in Figure2.

Mass culture

Laboratory culture of *R.robini* was established from field collected saffron corms before the start of the experiment. Corms were regularly monitored for laying of eggs (Figure ~~.~~3~~.~~ H). A single female laid up to approximately 100eggs each being dropped singly on the surface of the bulb.Adults were two days old when the mating began on the corms (Diaz~~.,~~ et~~.~~al. 2000). Some males died shortly after mating as was prominent from their colour change. Adults emerged from the month of September and remained alive ~~upto~~up to November.

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Immature development

Egg

In the present study, twenty eggs laid by each female were lifted carefully with the help of moistened zero size camel brush and shifted on the fresh corm in ~~P~~petriplates to study the biology. Cotton beds were moistened with sufficient water to keep the corms moist. The eggs were white and translucent (Figure

3A). The hatching period was observed from four to ten days. The mean hatching period of eggs in August, September, October and November was worked out to be 6.8, 4.6, 3.3 and 9.96 days respectively with a mean of 6.16 days.

Larva

A six-legged larva emerged from the egg and the lived in this condition for 3.60 to6.91 days. After hatching the larvae was 0.15-0.2 mm long, ovoid in shape with three pair of legs and lacked genital suckers. The larval period was of 5.9, 4.5, 3.6 and 6.91 days in August, September, October and November respectively. With a mean of 5.22 dayslarvae molted into another stage with eight legs.

Table 3. Time taken (in days ± SD) taken by *Rhizoglyphus robini* during different months of the year for the completion of development.

Stage	August*	September*	October*	November*	Range	(Mean ± SD)
Egg	6.8± 0.43	4.6 ± 0.61	3.3± 1.43	9.96± 0.67	3.30-9.96	6.16 ±0.78
Larva	5.9± 0.49	4.5± 0.97	3.6± 0.76	6.91± 0.52	3.60-6.91	5.22± 0.68
Protonymph	6.6± 0.23	5.8 ± 0.21	3.03± 0.21	7.61± 1.43	3.03-7.61	5.76± 0.52
Tritonymph	7.3± 1.32	6.3± 0.22	4.71± 0.44	9.71± 1.68	4.71-9.71	7.00± 0.91
Adult	--	21.2± 1.32	14.64±0.99	32.1± 0.47	14.64-32.1	22.64± 0.92
Male	--	24.6± 0.43	22.5± 0.41	28.6± 0.49	22.50-28.6	25.23± 0.44
Female	--	22.2± 0.87	18.3± 0.55	24.2± 0.66	18.30-24.2	21.56± 0.69

* Mean of three replications.

Protonymph

The protonymph wassame as larvae with four pair of legs measuring 0.5 mmin length. The protonymph period in August, September, October and November was found to be 6.6, 5.8, 3.03 and 7.61 days respectively with a mean of 5.76 days. This stage can be distinguished from the tritonymph by having two genital suckers, whereas the tritonymph have three or four suckers (Figure 3B). Hypopal stage or heteromorphic deutonymph is anotherquiescent stage in bulb mitewhich appears when there is overcrowding or the substrate becomes too polluted by decay.Deutonymph stage can be induced by low food quality and quantity, high concentrations of waste products, and an extreme in temperature and humidity. This stage was not found during the study as the corms were changed regularly and as also reported by Diaz, et al.(2000).

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Tritonymph

This stage wasmilky white in colour and measuring 0.5 -0.6 mm in length with four pair of legs. This stage lasted for 7.3, 6.3, 4.71 and 9.71 days in August, September, October and November respectively with a mean of 7.0 days. In this stage abdomen was observed to be same as in the adult but the genitalia were undeveloped. The mites were bigger and bulged in size as compared to deutonymph (Figure 3C). This finding is in agreement with the observations of Woodring (1969) and Gerson et al. (1983), and Diaz et al. (2000).

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Adult

Mature bulb mites vary from 0.6 to 0.9 mm long and had four pairs of legs. Their bodies were shiny white.Initially 1-2 eggs were laid by adult and later increased in number up to 6-8. The mean number of eggs laid by females ranged from 100 to 120 eggs. The male adult survived for 24.6, 22.5 and 28.6 and female adult lasted for 22.2, 18.3 and 24.2 days in the months of September, October and November respectively (Figures 3E and 3G). The mean longevity of adult male was approximately 25.23days measuring about 0.50-0.60 mm long, whereas mean longevity of adult female was 21.56days and measured about 0.45-0.95 mm long. The total life cycle from egg to adult was approximately46.78 days. The various stages of *R. robini*, have a soft cuticle that expands during feeding. In the present study, relative humidity ranged from 70 to 75 percent during different months and had no impact on the developmental rate of the mite. These findings are in line with the results of Capua and &Gerson (1983) and Diaz et al. (2000)who reported that relative humidity has no impact on the development.

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The life cycle tends to be less active when there is drop in temperature,but they do not undergo a resting period, and all life stagesmay be present throughout the growing

fungal pathogens such as *Fusarium* spp. indicating that infected bulbs create conditions that are

favorable for bulb mite development as infested corms have exposed outer skin layers for easy entry of mites into the basal plate.

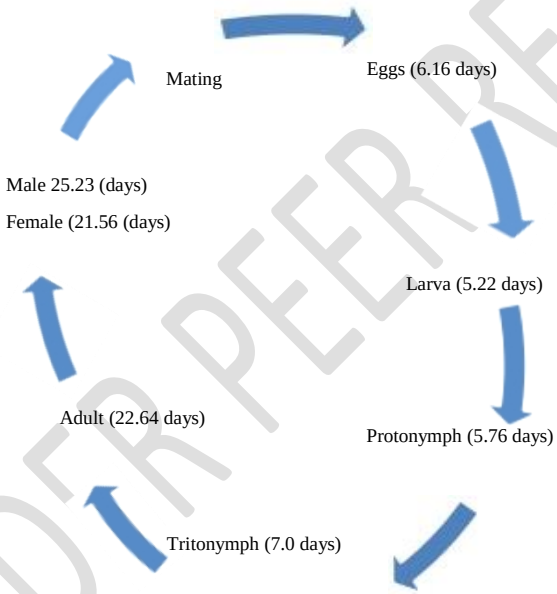

Figure -2.Diagrammatic representation of biology of bulb mite.

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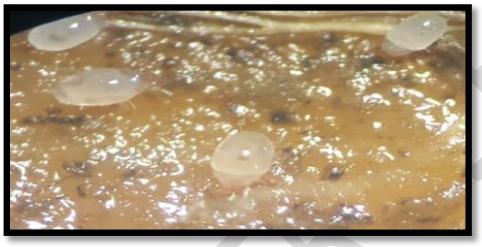
A. Eggs of bulb mite

B. Protonymph of Bulb mite

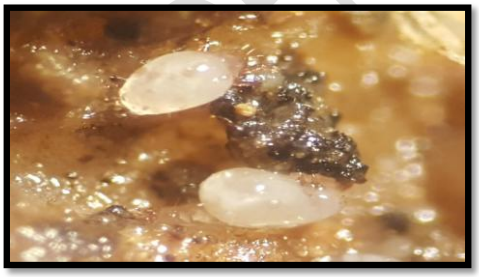
C. Tritonymph of Bulb mite

D. *Hypoaspisaculeifer*in association with bulb mite



E. Adults of Bulb mite

F. *Hypoaspisaculeifer* inside saffron corm



G. Adults of Bulb mite

H. Biology study of bulb mite in Laboratory

Figure3. Various life stages of bulb mite *Rhizoglyphus robini* on Saffron Cormsand *Hypoaspisaculeifer* inside saffron corm.

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Interaction of bulb mite with predatory mite *H. aculeifer*

The mesostigmatic soil dwelling predatory mite *H. aculeifer* was found associated with the bulb mite, *R. robini*during the study period (Figures ~~,~~3D and 3~~,~~F). The predatory mite was metallic brown in colour. They are agile as compared to its host *R. robini*which is slow moving. These predators feed and reproduce on the corms on which *R. robini*had already fed. The nymph and adult stages of *H. aculeifer* feed on all life stages of *R. robini*. The ability of *H. aculeifer* to suppress or regulate bulb mites depends on population density and exposure to the predatory mite (Lesna et al. 2000). Bulb mites may hide and establish in the inner layers of bulbs which may be more difficult for the predatory mite to locate but because of its active nature it can search the hidden bulb mites as well.

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Management of Saffron bulb mite

The results of the experiment conducted at SKUAST-K Shalimar duringthe years 2019, 2020 and 2021 where efficacy of different doses of acaricides were evaluated againstbulb mite. The treatments and their combinations with other methods is depicted in table 4. Perusal of the data in the table 4revealed that lowest amount of stunted growth of leaves and chlorosis (4.23~~percent~~%), highest emergence of saffron flower (97.34 ~~percent~~%) and lowest bulb rot (2.23~~percent~~%) was observed in T5 in which method of application was drenching the soil with chlorpyrifos 20 EC+ foliar spray with cyenopyrafen30 SC @ 3.0and 1.0 ml water litre^{-1} respectively. This was followed by T3 in which 8.32 percent with stunted growth of leaves and chlorosis, 93.41~~, percent~~% emergence of saffron flower and 3.67 percent bulb rot was observed where the method of application was soaking bulbs in imidacloprid 70 WG before planting + foliar spray with cyenopyrafen 30 SC @ 1.5g+1.0 ml water litre^{-1} respectively.This was in turn followed by T2 where 11.98, 83.12 and 6.21 percent stunted leaves and chlorosis, emergence of saffron flower and bulb rot respectively was recordedwherein method of application was drenching the soil with chlorpyrifos 20 EC @ 3.0 ml water litre^{-1}. The highest percentage of stunted leaves and chlorosis, emergence of saffron flower and bulb rot were in T4 with 16.11, 71.19 and 12.54respectively where the method of treatment included soaking bulbs in imidacloprid 70 WG before planting + foliar spray with propargite 57 EC @ 1.5g+1.0 ml water litre^{-1}respectively.All the treatments were significantly different over control when tested at p= 0.05. In treatment T7 (control) the amountof stunted leaves and chlorosis, emergence of saffron flower and bulb rot were 84.32, 09.94 and 65.54%~~, percent~~ respectively.

Table 4. Efficacy of different treatment strategies for the management of saffron bulb mite on saffron during the year 2019-2021

	Treatments	Method of application	Dose water^{-1}	Active substance mg water litre^{-1}	*Mean Stunted leaves and chlorosis (%)	*Mean Emergence of saffron flower (%)	*Mean Bulb rot (%)
T1	Imidacloprid 70 WG	Soaking bulbs in Imidacloprid before planting	1.5 g	1.05	13.09	77.21	9.82
T2	Chlorpyrifos 20 EC	Drenching the soil with Chlorpyrifos	3.0ml	0.6	11.98	83.12	6.21
T3	Imidacloprid 70 WG + Cyenopyrafen 30 SC	Soaking bulbs in Imidacloprid before planting + Foliar spray with Cyenopyrafen	1.5g + 1.0ml	1.05+0.3	8.32	93.41	3.67
T4	Imidacloprid 70 WG + Propargite 57 EC	Soaking bulbs in Imidacloprid before planting + Foliar spray with Propargite	1.5g + 1.0ml	1.05+0.57	16.11	71.19	12.54
T5	Chlorpyrifos 20 EC + Cyenopyrafen 30 SC	Drenching the soil with Chlorpyrifos + Foliar spray with Cyenopyrafen	3.0ml + 1.0ml	0.6+0.3	4.23	97.34	2.23
T6	Chlorpyrifos 20 EC + Propargite 57 EC	Drenching the soil with Chlorpyrifos + Foliar spray with Propargite	3.0ml + 1.0ml	0.6+0.57	14.98	73.42	10.84
T7	Control		---	---	84.32	09.94	65.54
	CD (p=0.05)				1.29	2.97	0.79

*Mean of three replications

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Discussion

Saffron bulb mite *Rhizoglyphus robini* is emerging as one of the most destructive insect pests of saffron under agro ecological conditions of Kashmir. The present study was aimed to study the level infestation of this pest at various locations, its biology and management under temperate conditions of Kashmir. Available literature on the level of infestation of this mite in Kashmir is scare and hence could not be correlated with the literature. Biology of the mite was studied under room temperature of 25± 5^0Cin the laboratoryfrom August to November duringthe years 2019, 2020 and 2021. The life cycle of *R. robini* are egg, larva, protonymph, tritonymph and adult (Houck and O Connor 1991). The life cycle is influenced by temperature and food quality (Diaz, et al. 2000). *R. robini* strictly follows sexual reproduction (Woodeing, 1969; Gerson et al. 1983).

The development rate of various stages of the mite slowed down when ambient temperature of the laboratory decreased below the optimum temperature. The mean duration taken by the mite to complete various life stages were egg (6.16), Larva (5.22), protonymph (5.76), tritonymph (7.00), adult male (25.23) and adult female (21.56) days. Development of immature stages was found to be quick during August to October when the temperature remains high, but slows down during November when there is significant drop in temperature. The findings are in agreement with Gerson et al. (1983); Fashing and Hefele (1991); Raut and Sarkar (1991).Feeding wounds created by bulb mites on corms provide entry sites for soil borne fungal pathogens such as Pythium, Rhizoctonia, and Fusarium. The attractancy of *R. robini*to the fungal pathogens has been reported by several workers. Corms of Gladiolus infested by various fungi and bacteria attracted bulb mite as described by Noble and Poe (1972).Rakkyo bulbs infested with Fusarium were attracted to *R. robini* as described by Shinkaji et al. (1988a). Extracts from Fusarium culture filtrates upon identification revealed that alcohols (ethanol, n-propanol, iso- butamol, iso-penthanol, and 2-methyl-1-butanol) acted as mite attractants in vitro (Shinkaji et al. 1988b).

Various treatments and different methods of application were tested for the control of bulb mite. The method of application included drenching the soil, soaking the bulbs and foliar application with different pesticides. Among all the treatments,drenching of the soil with chlorpyrifos 20 EC followed by foliar spray with cyenopyrafen30 SC @ 3.0 and 1.0 ml water $litre^{-1}$ respectively proved to be the best treatment in terms of reduction in stunted leaves and chlorosis, increase in flower emergence and reduction in bulb rot whereas soaking bulbs in imidacloprid 70 WG before planting followed by foliar spray with propargite 57 EC @ 1.5g and 1.0 ml water $litre^{-1}$ respectively worked out to be least effective in reducing the plant damage. Chlorpyrifos is an organophosphate insecticide and served as a fumigant in soil in minimizing the mite population. Cyenopyrafen is effective against all the stages of mites. It has quick action and kills mites with short time (Bajya and Ranjith 2016). Fumigation has been observed to be

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effective against bulb mites (Zhao et al.1996)

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Comment [CES19]: The manuscript does not presents any conclusion. It must have a presentation of the results and do not describe the main importance of the research.
It needs a serious major revision.
What is the main conclusion of the research and the impact of the knowledge of the results to society and science?

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Comment [CES23]: Very significant number of old references.

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