Proteinex Bait Gel Formulations Suitable for increased Field Stability and Enhanced Trapping of Melon fly, *Zeugodacus cucurbitae* Coquilette

ABSTRACT

Cucurbit fruit fly, *Zeugodacus cucurbitae* is an important insect pest of cucurbits. Fruit flies consisted of over 250 species worldwide and its maggots damage fruit from inside and hence, insecticides remain practically ineffective. Trapping the adult flies through parapheromenes, protein and food based traps is the most common practice. The protein and food baits attract both sexes over the parapheromones and are more effective due to their attractiveness and comparatively less weathering under field conditions. Hence, the present study was undertaken to prepare gel based formulations of liquid proteinex bait to increase it's trapping efficiency. The gelling agents *viz.,* xanthan gum powder, gaur gum powder, pectin powder, carrageenan powder, sodium alginate and gum arabic powder were used to prepare gel formulations of liquid proteinex bait developed by us. Among them, pectin and xanthan gum powders turned the liquid proteinex bait to semi-liquid at very less concentrations thus making them economical to use. Pectin, guar gum and carrageenan powder gel formulations maintained semi-liquid to slightly thickened consistency up to 9th day after preparation of bait and is advantageous for stability under field conditions. Protein content of pectin and guar gum powder formulations are similar as liquid proteinex bait which contributes for more attraction. pH of pectin and guar gum powder gel formulations was higher than the liquid proteinex bait thus increasing their attractiveness. As viscosity of pectin and carrageenan powder formulations was close to liquid proteinex bait, acceptability of these baits closely follows that of liquid bait. Study concluded that pectin powder gel formulation of liquid proteinex bait was found to be ideal and economical to use in field conditions to attract both male and female fruit flies.

Keywords: *Zeugodacus cucurbitae* – Proteinex bait – Gel formulation – Pectin powder – Guar gum powder – Carrageenan powder

1. INTRODUCTION

Fruit flies are the highly polyphagous insect pests with a worldwide distribution. Among the fruit fly species, over 250 are economically important and can be seen throughout the world in temperate, subtropical and tropical climates (Christenson and Foote, 1960) with the exception of extreme desert and polar regions, where their hosts are limited or do not exist (Foote *et al.,* 1993). De Meyer *et al.* (2015) stated that Asia is the origin of melon fruit fly, *Zeugodacus cucurbitae* Coquilette and India is generally considered as its native home. *Z.cucurbitae* is a versatile species of fruit fly and associated with over 140 host plants. It can withstand adverse climatic conditions in plains and hilly areas (Kapoor, 2002). *Z.cucurbitae* is oligophagous (Fletcher, 1987). Doharey (1983) reported the preference of *Z.cucurbitae* to cucurbit hosts *i.e.,* bitter gourd, musk melon, snap melon and snake gourd in India. Agarwal & Sueyoshi (2005) and Satarkar *et al.* (2009) reported the fruit flies as one of the most destructive insect pests of horticulture crops throughout the world. Among the fruit

fly species, *Z.cucurbitae* was considered as a major problem to cucurbit crops as it causes heavy damage to fruits and vegetables in Asia (Nagappan *et al.,* 1971).

Fruit flies attack a variety of cucurbitaceous crops causing direct fruit loss and also causes indirect damage making the fruits vulnerable to the colonization of secondary saprophytes (Ordax *et al.,* 2015). Several measures have been documented to reduce the infestation of fruit flies in vegetables and fruit production. Insecticide application is one among them but not effective since, the insect has hidden life stages (Dhillon *et al.,* 2005). Moreover, pesticide residues in cucurbit vegetables are of major concern (Gogi *et al.,* 2010). This was evident with the rejection of several consignments of exports of fruits and vegetables due to pesticide residue issues (Baig *et al.,* 2009; Praveen *et al.,* 2012). In this context, trapping of fruit flies by employing baits in field conditions is the most practical management strategy for fruit flies.

As parapheromones available currently are able to attract only male fruit flies and this trapping system that targets female fruit flies was considered more effective as it prevents oviposition causing less crop damage (Siderhurst and Jang, 2010). Andrew W. McCracken (2023) also reiterated that Protein-bait traps can attract both sexes of fruit flies and hence exert a greater long-term impact on insect populations by decreasing the reproductive potential of trapped individuals. They also stated that, even though, cuelure had overall higher trapping performance, protein-bait traps, despite trapping at lower efficiency, were equally effective for trapping males and females and have an inclusive advantage by trapping females and thereby preventing future individuals.

Based on our previous research studies (laboratory and field experiments) on effectiveness of several protein and food baits to cucurbit fruit flies, liquid proteinex bait was found to be the most luring to *Z.cucurbitae* (Sruthi *et al.,* 2021). However, exposure of liquid baits in field conditions made the baits dry and decreased its attractiveness. To address this problem, the present study was undertaken to prepare gel based bait formulations to prevent the evaporation of water from the baits and in turn to increase the bait attractiveness at Agricultural College and Research Institute, Madurai, Tamil Nadu.

2. MATERIALS AND METHODS

2.1. Preparation of gel formulations of proteinex bait

A total of twelve gelling agents or hydrocolloid thickeners *viz.,* agar powder, agar strands, gelatine powder, carrageenan powder, corn starch powder, gaur gum powder, xanthan gum powder, sodium alginate, wheat starch powder, pectin powder, gellan gum

powder and gum arabic powder were selected to add to proteinex bait to form gel formulations with semiliquid consistency.

Based on the previous literature and on trial & error basis, three doses of the selected gelling agents were mixed in the proteinex bait to prepare the gel formulations.

Table 1. Test dosages with references of gelling agents

S. No.	Gelling agent	Test doses (%)			Reference
		Dose 1	Dose 2	Dose 3	
1	Agar powder	0.5	1.0	1.5	Gillespie (1993)
2	Agar strands	0.5	1.0	1.5	Armisen *et al*. (2009)
3	Gelatine powder	0.5	1.0	1.5	On Trial and error basis
4	Carrageenan powder	3.0	3.5	4.0	On Trial and error basis
5	Corn starch powder	2.0	2.5	3.0	On Trial and Error basis
6	Gaur gum powder	2.0	2.5	3.0	Jantrawut and Ruksiriwanich (2014)
7	Xanthan gum powder	0.5	1.0	1.5	On Trial and Error basis
8	Sodium alginate	1.0	1.5	2.0	Debanath *et al.*(2011)
9	Wheat starch powder	2.0	2.5	3.0	On Trial and Error basis
10	Pectin powder	0.5	1.0	1.5	Kakino *et al.* (2017)
11	Gellan gum powder	1.0	2.0	3.0	On Trial and Error basis
12	Gum Arabic powder	7.0	7.5	8.0	On Trial and Error basis

2.2. Preparation of gel formulations of proteinex bait without heating

Among the selected gelling agents, xanthan gum powder, gaur gum powder, pectin powder, carrageenan powder, sodium alginate and gum arabic powder are able to dissolve in water without heating. Three test doses of each gelling agent were added with 100 ml of proteinex bait separately and mixed thoroughly using an electric blender for 3 minutes. Then they were poured into three different containers and allowed to form the gel consistency.

2.3. Preparation of gel formulations of proteinex bait with heating

The gelling agents *i.e.*, agar powder, agar strands, gelatin powder, corn starch powder, wheat starch powder and gellan gum powder needed heating for adding in proteinex liquid bait. Three doses of the above gelling agents were added in 10 ml of distilled water and heated in a microwave oven for 3 minutes. The boiled solution was kept at room temperature to turn to luke warm, later added to 90 ml of proteinex liquid bait and allowed to cool to form

the gel consistency. These gel formulations have not turned into semiliquid consistency and gel powders were separated as a layer in 20 to 30 minutes. So, the above gelling agents were eliminated from further testing.

2.4. Standardization of the optimum doses of gelling agents

After the gel formulations prepared without heating have turned to semi liquid state, their homogeneity and gel consistency were evaluated based on the tactile perception. Among the three gel formulations prepared from three test doses of each gelling agent, one with semiliquid consistency was selected and kept for observation. However, they retained the semi-liquid consistency up to five days only and hence re-standardization of doses was done.

2.5. Re-standardization of the selected test doses of gelling agents

To increase the retention time of semiliquid consistency, the selected dose of each gelling agent was re-standardized by lowering the dose to 0.2 g twice from the selected dose (if the selected dose is 2%, re-standardized test doses are 1.8% and 1.6%). Among these three doses, one dose which turned the liquid bait to semiliquid and able to retain that semiliquid consistency up to fifteen days (the maximum period of attraction of proteinex bait is 15 days) in field conditions was selected as the optimum dose.

2.6. Evaluation of quality parameters of final proteinex bait gel formulations

It is very important to retain the quality parameters of liquid proteinex bait even after adding gelling agent. Hence, the following quality parameters were estimated in the final gel formulations of proteinex bait.

1. Protein content was estimated using the micro Kjeldahl method.
2. pH was recorded with the digital portable pH meter.
3. Viscosity: This was determined using a viscometer (Brookfield viscometer) (Spindle no. 62 at 60 rpm and 100 rpm and 25°C). Gelation time (time required for changing the liquid bait to semiliquid consistency after adding gelling agent) was also noted.
4. Spreadability test:The gel formulation (0.5 g) was placed in the middle of a glass slide (25.4 x 76.2mm), another glass slide was kept on it and a weight of 250 g was placed on them. This setup was kept as such for 5 minutes after which, the length to which the gel formulation has spread was measured.

2.7. Statistical Analysis

Data were subjected to appropriate transformations before analysis. Means were separated by Tukey's Honestly Significant Difference (HSD) test. Statistical analyses were performed using Statistical Package for the Social Sciences Windows (version 22.0) (IBM Corp. Released 2013) (Gomez and Gomez, 1984).

3. RESULTS AND DISCUSSION

3.1. Standardization of the optimum doses of gelling agents to add to liquid proteinex bait

Three test doses of each gelling agent were added to the liquid proteinex bait and the test dose which gave a semi-liquid consistency to the liquid bait was selected as the standardized dose. The selected standardized doses of carrageenan powder, gaur gum powder, xanthan gum powder, sodium alginate powder, pectin powder and gum arabic powder are 4.0%, 2.0%, 1.0%, 2.0%, 1.0% and 7.5% respectively (Table 2).

3.2. Re-standardization of the doses of gelling agents to add to liquid proteinex bait

Standardized doses of gelling agents which gave semi-liquid consistency to the liquid proteinex bait were again re-standardized to maintain the semi-liquid consistency for more number of days in field conditions. Among the three test doses, re-standardized doses of gelling agents selected were 3.6%, 1.6%, 0.6%, 1.8%, 0.8% and 7.3% for carrageenan powder, gaur gum powder, xanthan gum powder, sodium alginate powder, pectin powder and gum arabic powder respectively (Table 2).

Table 2. Standard test and re-standardized doses of gelling agents

S. No.	Gelling agent	Standardization				Re-standardization			
		Test dose 1 (%)	Test dose 2 (%)	Test dose 3 (%)	Standardized dose (%)	Test dose 4 (%)	Test dose 5 (%)	Test dose 6 (%)	Re-standardized dose (%)
1	Carrageenan powder	3.0	3.5	4.0	4.0	3.6	3.8	4.0	3.6
2	Gaur gum powder	2.0	2.5	3.0	2.0	1.6	1.8	2.0	1.6
3	Xanthan gum powder	0.5	1.0	1.5	1.0	0.8	0.6	1.0	0.6
4	Sodium alginate	1.0	1.5	2.0	2.0	1.6	1.8	2.0	1.8
5	Pectin powder	0.5	1.0	1.5	1.0	0.6	0.8	1.0	0.8
6	Gum Arabic powder	7.0	7.5	8.0	7.5	7.1	7.3	7.5	7.3

Among the twelve gelling agents, heating process was needed for adding agar powder, agar strands, gelatin powder, corn starch powder, wheat starch powder and gellan

gum powder in proteinex liquid bait. Inspite of heating, mixing was not possible and gel powders were separated as a layer. Hence, rest of the gelling agents *viz.*, xanthan gum powder, gaur gum powder, pectin powder, carrageenan powder, sodium alginate and gum arabic powder which were able to dissolve in bait without heating were tested.

The re-standardized doses of gelling agents were as, xanthan gum powderand pectin powder are able to form semi-liquid proteinex bait at very less concentrations i.e., 0.6% and 0.8% respectively. More amounts of gum arabic powder (7.3%) and carrageenan powder (3.6%) are needed to add to make liquid proteinex bait semi-liquid. For the same purpose, the required quantity of gaur gum powder and sodium alginate is less *i.e.*, 1.6% and 1.8% respectively.

Utility of using gelling agents has been documented as, carrageenan is extracted from selected species of red seaweed and is a natural hydrocolloid with unique functionalities. It is used in a wide variety of applications in the food industry as a thickening, gelling, stabilizing and suspending agent in water and milk systems. Guar gum is a galactomannan polysaccharide extracted from guar beans and has thickening and stabilizing properties useful in food, feed, and industrial applications. Pectin powder is used as a thickener in cooking and baking. Xanthan gum is a common food additive made from sugar fermented by bacteria. It acts as a binder, thickener and stabilizer to improve texture and keep ingredients from separating. It also acts as an emulsifier.

Carbohydrates are the most frequently used gelling agents in food which includes gums such as guar gum and carboxymethylcellulose, carrageenan, agar, starch, alginates and pectins (Cohen, 2003). Gelling agents are essential components in insect diets because they keep water in a solid state, avoid interactions between ingredients and preserve the blended condition of the ingredients. According to Kaakeh *et al.* (1997) and Appel and Tanley (2000), most of the insect diets comprise approximately 3.0% agar and gel diets are proven to be convenient to use and highly effective.

Gelling agents used in the current study are also used in the food industry mostly as additives to thicken and stabilize foods by forming a gel. They are used in many foods, including desserts, jellies, candies, salad dressings, and yogurts. But up to now there are no reports of using these hydrocolloids to change the consistency of fruit fly baits to increase the keeping period in field conditions. Our study is an unique attempt towards this direction.

3.3. Evaluation of changes in consistency of gel formulations of proteinex bait

Among the gel formulations of proteinex bait, semi-liquid consistency was observed up to the maximum of 5 days after preparation (DAP) in pectin powder and guar gum powder

gel formulations (Table 3). In xanthan gum powder, carrageenan powder, sodium alginate gel formulations, semi-liquid consistency was observed up to 4 DAP and in gum arabic powder gel formulation, it was only 3 DAP. In pectin powder, guar gum powder and carrageenan powder gel formulations, moderately thickened consistency started only after 9 DAP and it is a suitable quality to increase the bait's practical utility in field conditions by reducing the evaporation. Among these three, the quantity of powder required to add in the liquid bait was less in pectin powder followed by guar gum powder and carrageenan powder. Thus pectin powder is economical to use by the farmers. However, to know the effects of adding these gel powders in liquid bait, studies were conducted on quality parameters of these gel formulations.

Table 3. Changes in the consistency of gel based formulations of protinex bait

S. No.	Gel based formulation	Days after preparation														
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
1	Xanthan gum powder gel formulation	+	+	+	+	++	++	++	++	+++	+++	+++	+++	++++	++++	++++
2	Carrageenan powder gel formulation	+	+	+	+	+	++	++	++	++	+++	+++	++++	++++	++++	++++
3	Pectin powder gel formulation	+	+	+	+	+	++	++	++	++	+++	+++	+++	+++	++++	++++
4	Sodium alginate gel formulation	+	+	+	+	++	++	++	+++	+++	+++	+++	+++	+++	++++	++++
5	Guar gum powder gel formulation	+	+	+	+	+	++	++	++	++	+++	+++	+++	+++	++++	++++
6	Gum arabic powder gel formulation	+	+	+	++	++	++	++	+++	+++	+++	+++	++++	+++	+++	+++

Semiliquid (+) Slightly thickened (++) Moderately thickened (+++) Highly thickened (++++)

3.4. Studies on quality parameters of gel formulations of liquidproteinex bait

3.4.1. Protein content

Protein content of the gel formulations was compared with the liquid proteinex bait to assess any variations in their protein content due to the addition of gelling agents. Among the various formulations, protein content was high in the liquid proteinex bait (27.56%). Next to this, guar gum powder and pectin powder gel formulations recorded 26.67 and 25.59 per cent protein contents, respectively (Table 4) and were at par with the liquid proteinex bait. Among the gel formulations, in xanthan gum powder gel formulation, less protein content (22.58%) was recorded. Among the various gel formulations, guar gum and pectin powder formulations recorded more or less similar protein content as liquid proteinex bait which is an added advantage to these formulations.

Several reports are available on the importance of protein to fruit fly multiplication as they are essential for their reproductive success. When males of *Bactrocera tryoni* were fed on protein rich diet, their general activity *i.e.*, mating success and longevity were increased. *B.tryoni* females when mated to protein fed males, stored sperms longer than the females mated to protein deficient males (Fanson *et al.*, 2009; Prenter *et al.*, 2013).

3.4.2. pH

Among the gel formulations of proteinex bait, more pH was recorded in the guar gum powder (7.27) and pectin powder gel formulations (7.26) and this was higher than that of the liquid proteinex bait (7.15). In xanthan gum powder gel formulation, slightly higher pH (7.20) than the proteinex bait was observed but both were on par with each other (Table 4). Carrageenan, gum arabic and sodium alginate gel formulations recorded less pH than the proteinex liquid bait. pH of the bait is very critical for their attractiveness. In that sense, among the proteinex bait gel formulations, in guar gum powder, pectin powder and xanthan gum powder gel formulations, pH *i.e.*, 7.27, 7.26 and 7.20, respectively was higher than the liquid proteinex bait (7.15) which is an added advantage to these gel formulations.

Previous reports of Bateman and Morton (1981) confirmed the attractiveness of standard yeast protein hydrolysate mixturewith increase in pH to the Queensland fruit fly, *Dacus tyroni*. Mazor *et al.* (1987) stated that increase in the attraction of protein-based baits at elevated pH levels is due to the increased ammonia release which is attractive to the female fruit flies and release of other volatiles on basification of the bait.

Ghanim and Metwally (2019) reported that attraction of females of *Ceratitis capitata* was influenced more by pH-level than concentration percentage of the solution and the more attraction was found at comparatively higher pH value. Sruthi *et al.*

(2021) tested food baits with tomato, banana, bitter gourd, pineapple, guava as main ingredient against melon fruit fly, *Z.cucurbitae* in bitter gourd and they reported that gradual increase in pH of baits increased the attraction to fruit flies.

3.4.3. Viscosity

Viscosity of gel formulations of liquid proteinex bait was compared with the viscosity of liquid proteinex bait. Viscosity of gel formulations ranged from 0.018 to 0.187 Pa.s (pascal- second) at 100 rpm and 0.017 to 0.275 Pa.s at 60 rpm. At 60 rpm, carrageenan powder gel formulation had the viscosity (0.017 Pa.s) closer to that of liquid protinex bait (0.005 Pa.s) followed by gum arabic gel formulation (0.033 Pa.s) and pectin gel formulation (0.061 Pa.s). Among the gel formulations, sodium alginate gel formulation (0.275 Pa.s) and xanthan gum powder gel formulation (0.194 Pa.s) were highly viscous (Table 4). As viscosity increases acceptability of bait to fruit flies decrease. Viscosity of carrageenan, gum arabic and pectin powder gel formulations was closer to that of liquid proteinex bait and hence addition of these gelling agents does not alter the attractiveness of these formulations.

Table 4. Estimation of quality parameters of gel based formulations of proteinex bait

S. No.	Formulation	Protein content (%)	pH	Viscosity of gel based formulation (Pa.s)*	
				100 rpm	60 rpm
1	Proteinex liquid bait	27.56 (31.66)^{a}	7.15 (2.67)^{ab}	0.006 (0.077)^{a}	0.005 (0.071)^{a}
2	Xanthan gum powder gel formulation	22.58 (28.37)^{d}	7.20 (2.68)^{ab}	0.109 (0.330)^{f}	0.194 (0.440)^{f}
3	Carrageenan powder gel formulation	24.25 (29.50)^{bcd}	6.91 (2.63)^{bc}	0.018 (0.136)^{b}	0.017 (0.129)^{b}
4	Pectin powder gel formulation	25.59 (30.38)^{abc}	7.26 (2.69)^{a}	0.051 (0.227)^{d}	0.061 (0.248)^{d}
5	Sodium alginate gel formulation	23.62 (29.08)^{cd}	6.95 (2.64)^{abc}	0.187 (0.433)^{g}	0.275 (0.525)^{g}
6	Guar gum powder gel formulation	26.67 (31.09)^{ab}	7.27 (2.70)^{a}	0.057 (0.238)^{e}	0.070 (0.265)^{e}
7	Gum arabic powder gel formulation	23.75 (29.16)^{cd}	6.65 (2.58)^{c}	0.034 (0.183)^{c}	0.033 (0.183)^{c}
	CD (0.05)	2.17	0.08	0.059	0.058
	S.E(d)	0.52	0.02	0.00036	0.00135

Pa.s – pascal seconds

*Mean of three replications

Figures in parentheses are arc sine transformed values

Means followed by the same letter in a column are not significantly different by Tukey's HSD test. (P=0.05)

3.4.4. Spreadability

Among the gel formulations of liquid proteinex bait, spreadability was more in carrageenan powder gel formulation with increased diameter of 4.3 cm followed

by pectin powder &guar gum powder gel formulations (increased diameter of 4.1 cm) and sodium alginate gel formulation (increased diameter of 4.0 cm) (Table 5).

3.4.5. Gelation time

Among the gel formulations of proteinex bait, time required to form the semi-liquid consistency *i.e.,* gelation time was less in pectin powder gel formulation (1 to 2 minutes) followed by sodium alginate (2 to 3 minutes) and guar gum powder gel formulation (4 to 5 minutes) (Table 5).

Table 5. Spreadability and gelation time of gel formulations of proteinex bait

S. No.	Formulation	Spreadability (cm)			Gelation time (minutes)
		Initial diameter (I)	Final diameter (F)	Increased diameter (I -F)	
1	Xanthan gum powder gel formulation	2.9	6.8	3.9 (1.97)^{c}	6 - 8
2	Carrageenan powder gel formulation	2.1	6.4	4.3 (2.07)^{a}	5 - 8
3	Guar gum powder gel formulation	3.8	7.9	4.1 (2.02)^{b}	4 - 5
4	Sodium alginate gel formulation	3.2	7.2	4.0 (2.00)^{bc}	2 - 3
5	Pectin powder gel formulation	3.4	7.5	4.1 (2.02)^{b}	1 - 2
6	Gum arabic powder gel formulation	3.5	7.1	3.6 (1.90)^{d}	11 - 15
			CD (0.05)	0.05	
			S.E(d)	0.01	

*Mean of three replications

Figures in parentheses are square root transformed values

Means followed by the same letter in a column are not significantly different by Tukey's HSD test. (P=0.05)

4. CONCLUSION

Among the gelling agents *viz.,* xanthan gum powder, gaur gum powder, pectin powder, carrageenan powder, sodium alginate and gum arabic powder, comparatively at very less concentrations xanthan gum powder and pectin powder turned the liquid proteinex bait to semi-liquid. Moderately thickened consistency in pectin powder, guar gum powder and carrageenan powder gel formulationswas seen only after 9th day after preparation of bait and this is practically desirable and advantageous in field conditions. Among these three, pectin powder is able to form semi-liquid consistency with the addition of small quantity of powder in the liquid bait followed by guar gum powder and carrageenan powder. Thus pectin powder is economical to use by the farmers. With regard to the protein content, pectin and guar gum

powder formulations are observed with similar protein content as liquid proteinex bait which made these formulations equally attractive as liquid bait. Moreover, pectin, guar gum and xanthan gum powder gel formulations recorded more pH than the liquid proteinex bait adding more advantage to these gel formulations. Generally increased viscosity increases the acceptability of bait to fruit flies. As viscosity of pectin, carrageenan and gum arabic powder formulations was nearer to liquid proteinex bait, their addition to liquid bait doesnot alter the attractiveness of these formulations. To conclude, pectin powder gel formulation of liquid proteinex bait was found to be ideal and economical to use in field conditions to attract both male and female fruit flies. In this, spreadability is also optimum and gelation time is comparatively less.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that no generative AI technologies such as large language models (CHATGPT, COPILOT etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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