রেজিস্টার্ড নং ডি এ-১

বাংলাদেশ গেজেট

অতিরিক্ত সংখ্যা

কর্তৃপক্ষ কর্তৃক প্রকাশিত

মঙ্গলবার, আগস্ট ৩, ২০১০

গণপ্রজাতন্ত্রী বাংলাদেশ সরকার

কৃষি মন্ত্রণালয়

প্রজ্ঞাপন

তারিখ, ১৪ শ্রাবণ ১৪১৭ বঙ্গাব্দ/২৯ জুলাই ২০১০ খ্রিস্টাব্দ

এস, আর, ও নং ২৮২-আইন/২০১০।—যেহেতু সরকার The Pesticides Ordinance, 1971 (Ordinance No. II of 1971) এর Section 29 (1) এ প্রদত্ত ক্ষমতাবলে Pesticides Technical Advisory Committee এর সাথে পরামর্শক্রমে The Pesticide Rules, 1985 নিম্নলিখিতভাবে সংশোনের প্রস্তাব করিয়া প্রস্তাবিত সংশোধনীর ফলে প্রভাবিত বা ক্ষতিগ্রস্ত হইতে পারেন এমন সকল ব্যক্তিদের অবগতি এবং তাহাদের নিকট হইতে আপত্তি বা পরামর্শ আহবান করিয়া প্রস্তাবিত সংশোধনী গেজেটে প্রকাশের তারিখ হইতে ৩০ (ত্রিশ) দিনের সময়সীমা উল্লেখে উক্ত Section 29 (1) এর বিধান অনুযায়ী বাংলাদেশ গেজেটের অতিরিক্ত সংখ্যায় ২৬ বৈশাখ, ১৪১৭ বঙ্গাব্দ মোতাবেক ৯ মে ২০১০ খ্রিস্টাব্দ তারিখে প্রাক-প্রকাশ করিয়াছিল; এবং

যেহেতু, জারীকৃত প্রজ্ঞাপনের প্রেক্ষিতে কাহারও নিকট হইতে কোন আপত্তি বা পরামর্শ পাওয়া যায় নাই;

মূল্য ঃ টাকা ২৬.০০

সেহেতু, The Pesticides Ordinance, 1971 (Ordinance No. II of 1971) এর Section 29 (1) এ প্রদত্ত ক্ষমতাবলে সরকার Pesticides Technical Advisory Committee এর সাথে পরামর্শক্রমে The Pesticide Rules, 1985 এর নিম্নরূপ সংশোধন করিল, যথা ঃ—

উপরিউক্ত Rules এর—

(১) rule 2 এর clause (1) এর প্রান্তস্থিত (.) ফুল স্টপের পরিবর্তে (;) সেমিকোলন প্রতিস্থাপিত হইবে এবং অতঃপর নিম্নরূপ নতুন clause (m), (n), (o), (p), (q), (r) এবং (s) সংযোজিত হইবে, যথা ঃ—

"(m) **"Active ingredient"** means the biologically active part of the pesticide present in a formulation;

(n) **"Agricultural pesticide"** means the pesticides which are intended for use against agricultural pests;

(o) **"Biopesticide"** According to the FAO, "Biopesticides" are naturally occurring substances or their synthetic analogues that are distinguished from conventional chemical pesticides by their unique modes of action, low use volume, and target species specificity;

(p) **"Inert ingredient"** means an ingredient in a formulated pesticide product which will not prevent, destroy, repel or mitigate any pest and is intentionally included in the product and also includes ingredient such as solvents, emulsifiers, wetting agents, carriers, diluents, conditioning agents etc.;

(q) **"Manufacturer"** means a corporation or other entity in the public or private sector or any individual engaged in the business or function (whether directly or through an agent or through an entity controlled by or under contract with it) of manufacturing a pesticide active ingredient or preparing its formulation or product;

(r) **"Public health pesticide"** means the pesticides which are intended for use against public health concern;

(s) **"Person"** means person as defined in clause (mm) of section 3.";

(২) rule 3 এর পরিবর্তে নিম্নরূপ rule 3 প্রতিস্থাপিত হইবে, যথা ঃ—

"3. Application for registration of pesticides.—An application in triplicate for registration of a brand of pesticide under sub-section (1) of section 5 shall be made to the Registration Authority in FORM 1(a) for Chemical pesticides, FORM 1(b) for Biochemical pesticides and FORM 1(c) for Microbial pesticides.";

(৩) rule 4 এর পরিবর্তে নিম্নরূপ rule 4 প্রতিস্থাপিত হইবে, যথা ঃ—

"4. Registration of Pesticide.—(1) On receipt of an application for registration of a brand of a pesticide, the Registration Authority shall send the application together with a sample of pesticide to the laboratory for test or analysis and to ascertain whether the sample is in accordance with the information provided along with the application.

(2) On receipt of the result of the test or analysis under sub-rule (1) the Registration Authority shall submit the result to the sub-committee for approval and after getting approval of the sub-committee the Registration Authority shall send the sample of the pesticide to the respective specialized institutes or organization authorized by the Advisory Committee to conduct biological test and trial for both the new molecule and Me-too product under field condition as may be required.

(3) Both for Me-too Product & New Molecule, two different locations and two crop season trials are required and after field trial, report should be made available within 1(one) month.

(4) The respective specialized institutes or organization shall conduct such biological and field efficacy tests following standard Bio-efficacy Test Protocols either available with the respective specialized institutes or organization or prescribed by the Advisory Committee.

(5) The specialized institute or organization will send the test result to the Registration Authority and on receipt of the test result, the Registration Authority will send the result to the next meeting of the sub-Committee of the Advisory Committee.

(6) On receipt of the result the Sub-Committee will examine the test result with the list of protocols and shall send its recommendations to the Registration Authority within 15(fifteen) days.

(7) On receipt of the recommendations under sub-rule (6) the Registration Authority will forward the recommendations to the advisory committee and if advisory committee approves and recommends that the brand of pesticide conforms to the requirement of the Ordinance and rules then the Registration Authority will give registration to the brand of pesticide in form 2 in such condition as may be specified in the registration certificate.

(8) A certificate of registration granted under sub-rule (7) shall apply only to the pesticide described in the application to which the certificate relates.

(9) The Registration Authority shall issue the registration certificate within 15(fifteen) working days of Advisory Committee's approval.

(10) Pesticides registered as aforesaid shall be published by the Registration Authority in the Official Gazette within 30(thirty) days from the date of granting registration certificate.

(11) The registration of a brand of pesticide shall be effective from the date of its registration until the thirtieth day of June of the third year following the year of registration.";

(৪) rule 6 এর পরিবর্তে নিম্নরূপ rule 6 প্রতিস্থাপিত হইবে, যথা ঃ—

"6. Renewal of registration certificate.—(1) An application in duplicate for renewal of registration shall be made in Form 3 to the Registration Authority.

(2) The registration of a pesticide shall be deemed to have been cancelled if not applied under sub-rule (1).

(3) A certificate of renewal of registration shall be issued in Form 4 within 90(ninety) days after receiving the application.

(4) On receipt of application with fee the Registration Authority may approve the changes in address and country of origin of a registered brand of a pesticide.

(5) In case of change of coutry of origin, the sample of the product should be tested and should comply with the original product.";

(৫) rule 7 এর পরিবর্তে নিম্নরূপ rule 7 প্রতিস্থাপিত হইবে, যথা ঃ—

"7. Fees.—(1) A fee of Tk. 20,000.00 (Taka Twenty thousand) shall be paid with each application for a Registration Certificate and a fee of Tk. 5,000.00 (Taka five thousand) for renewal of certificate of registration, which shall, in no case, be refunded to the applicant.

(2) A fee of Tk. 2000.00 (Taka two thousand) shall be paid for a duplicate copy of the registration certificate if the original one is defaced, destroyed or lost."

(৬) rule 8 এ "Service" শব্দটির পরিবর্তে "Service and in the offical Gazette" শব্দগুলি প্রতিস্থাপিত হইবে;

(৭) rule 9 এ "Authority" শব্দটির পরিবর্তে "Authority and the Registration Authority will cancel the registration" শব্দগুলি প্রতিস্থাপিত হইবে;

(৮) rule 11 এর sub-rule (3) এর পর নিম্নরূপ sub-rule (4) সংযোজিত হইবে, যথা ঃ—

"(4) Pesticide shall be importable only from the manufacturer or formulator as mentioned in the Registration Certificate.";

(৯) rule 12 এর—

(অ) sub-rule (2) এ "FORM 12," শব্দ, সংখ্যা ও কমার পর "FORM 12(a)," শব্দগুলি, সংখ্যা ও কমা সন্নিবেশিত হইবে;

(আ) sub-rule (3) এর পরিবর্তে নিম্নরূপ sub-rule (3) প্রতিস্থাপিত হইবে, যথা ঃ—

"(3) An application for grant of license and renewal thereof under this rule will be accompanied by a fee specified below **:**

		License fee Taka	Renewal fee Taka
(a)	import	Two thousand	One thousand
(b)	manufacture or formulation	Five thousand	One thousand
(c)	holding in stock for wholesale	One thousand	Five hundred
(d)	retail sale	Three hundred	Two hundred
(e)	repacking	Two thousand	One thousand
(f)	pest control operation on commercial basis	Two thousand	Five hundred
(g)	Advertisement	One thousand	Five hundred "

(ই) sub-rule (5) এর পর নিম্নরূপ sub-rule (6) সংযোজিত হইবে, যথা ঃ—

"(6) Environmental certificate is required from Department of Environment for Pesticide repacker, formulation factory or manufacturer following Environmental conservation Act 1995 and Environmental Conservation Rules 1997."

(১০) rule 14 এ উলি□খিত "Tk. 25 (twenty five)" শব্দগুলি, সংখ্যা ও বন্ধনীর পরিবর্তে "Tk. 100 (one hundred)" শব্দগুলি, সংখ্যা ও বন্ধনী প্রতিস্থাপিত হইবে;

(১১) rule 15 এর sub-rule (2) এর পরিবর্তে নিম্নরূপ sub-rule (2) প্রতিস্থাপিত হইবে, যথা ঃ—

"(2) An application in duplicate for renewal of license shall be made to the Registration Authority within 30 (thirty) days of such expiry of the date of its effectiveness and if the application is not made so, a penalty of Tk. 300.00 (three hundred) for each month up to 3(three) months from the date of expiry, shall accompany the application along with usual renewal fee and the license of a pesticide shall be deemed to have been cancelled, if the renewal is not applied for within 90 (ninety) days from the date of expiry.";

(১২) rule 16 এর sub-rule (3) এ উল্লিখিত "৪৫-কৃষি প্রাপ্তি-অনিষ্টকারী পোকা-মাকড়, রোগ-বালাই ব্যবস্থাপনা ও বিবিধ খাতে আয়" শব্দগুলির পরিবর্তে "1-4331-0000-2043" সংখ্যাগুলি ও চিহ্নগুলি প্রতিস্থাপিত হইবে;

(১৩) rule 19 এর sub-rule (3) এর পরিবর্তে নিম্নরূপ sub-rule (3) প্রতিস্থাপিত হইবে, যথা ঃ

"(3) The licensing authority may, after such inspection finds all terms and conditions paid down under Rule 17 and/or which is applicable or fulfilled by the person to whom the license is proposed to be transferred, accord permission to transfer the license and on such permission being given, an endorsement to the that effect shall be made in the license.";

(১৪) rule 21 এর—

(অ) Clause (c) এ উল্লিখিত "pesticides" শব্দটির পরিবর্তে "Agricultural and Public Health pesticides" শব্দগুলি প্রতিস্থাপিত হইবে; এবং

(আ) Clause (d) এ উল্লিখিত "as well as their being suitable for aerial application" শব্দগুলির পরিবর্তে "based on formulation as well as they are being suitable for ground and aerial application" শব্দগুলি প্রতিস্থাপিত হইবে;

(১৫) rule 28 এর clause (d) এ উল্লিখিত "WHO" শব্দটির পরিবর্তে "WHO and FAO" শব্দগুলি প্রতিস্থাপিত হইবে;

(১৬) rule 32 এর—

(অ) "leaflet" শব্দের পর উল্লিখিত "of every pesticide which shall be affixed or attached to the package or repacking" শব্দগুলি বিলুপ্ত হইবে; এবং

(আ) Clause (d) এ উল্লিখিত "storage" শব্দটির পরে "shall be exclusive" শব্দগুলি সংযোজিত হইবে;

(১৭) rule 33 এর—

(অ) sub-rule (4) এর—

(i) Clause (i) এ "category 1 (highly toxic)" শব্দগুলি, সংখ্যা ও বন্ধনীর পরিবর্তে "1a and 1b (extremely and highly hazardous)" শব্দগুলি, সংখ্যা ও বন্ধনী প্রতিস্থাপিত হইবে;

(ii) clause (iii) এবং (iv) এর পরিবর্তে নিম্নরূপ clause (iii) এবং (iv) প্রতিস্থাপিত হইবে, যথা ঃ—

"(iii) pesticides in category II (moderately hazardous) shall bear the word "Poison" "DANGER" printed in bright yellow and the statement "KEEP OUT OF THE REACH OF CHILDREN" shall appear on the label at suitable out side the square;

(iv) pesticides in category III (slightly hazardous) shall bear the word "Poison" "CAUTION" and the word "CAUTION" printed in bright green and the statement "KEEP OUT OF THE REACH OF CHILDREN" shall appear on the label at suitable place outside the square.";

(আ) sub-rule (5) এর TABLE এর পরিবর্তে নিম্নরূপ TABLE প্রতিস্থাপিত হইবে, যথা ঃ

"TABLE

Class		LD 50 for the rat(mg/kg body weight)based on formulations				
		Oral		Dermal		Color of identification band on the label
		Solid	Liquids	Solids	Liquids	
1a	Extremely hazardous	5 or less	20 or less	10 or less	40 or less	Red
1b	Highly hazardous	5-50	20-200	10-100	40-400	Red
II	Moderately hazardous	50-500	200-2000	100-1000	400-4000	Bright yellow
III	Slightly hazardous	Over 500	Over 2000	Over 1000	Over 4000	Bright green

The terms "Solids" and "Liquids" refer to the physical state of the active ingredient being classified.";

(১৮) rule 38 এর sub-rule (2) এ "country" শব্দটির পরিবর্তে "country and FAO, WHO, ISO, CIPAC specification" শব্দগুলি ও কমাগুলি প্রতিস্থাপিত হইবে;

(১৯) rule 40 এর sub-rule (3) এ উল্লিখিত "৪৫-কৃষিপ্রাপ্তি-অনিষ্টকারী পোকা-মাকড়, রোগ-বালাই ব্যবস্থাপনা ও বিবিধ খাতে আয়" শব্দগুলি, সংখ্যা, চিহ্নগুলি ও কমার পরিবর্তে "1-4331-0000-2043" সংখ্যাগুলি ও চিহ্নগুলি প্রতিস্থাপিত হইবে;

(২০) rule 41 এর clause (c) এ "shall not exceed two pounds (one kilogram)" শব্দগুলি ও বন্ধনীর পরিবর্তে "shall be maximum 125ml for liquid, 500gms for granules and powder" শব্দগুলি, সংখ্যাগুলি ও কমা প্রতিস্থাপিত হইবে;

(২১) rule 58 এর clause (d) এরপর নিম্নরূপ নূতন clause (e) সংযোজিত হইবে, যথা ঃ—

"(e) ensure periodic monthly blood cholinesterase test for the workers and removing the worker from the plant, whose blood cholinesterase level has been depressed below WHO limits.";

(২২) rule 59 এর পরিবর্তে নিম্নরূপ rule 59 প্রতিস্থাপিত হইবে, যথা ঃ—

"59. Nomenclature of Plant and Animal life.—A list of the plant and animal life, the nomenclature of which are specified in Schedule II, shall be maintained at Plant Protection Wing (PPW) of the Department of Agricultural Extension and up-dated from time to time by the Director, PPW as per approval of the Advisory Committee.";

(২৩) rule 60 এর পরিবর্তে নিম্নরূপ rule 60 প্রতিস্থাপিত হইবে, যথা ঃ—

"60. Pesticides to be labeled poison.—(1) The pesticides which get registration are specified in Schedule III and shall be labeled "Poison" according to the provision of rule 33.

(2) Director, PPW shall update the list from time to time."

২৪। FORM 1 এর পরিবর্তে নিম্নরূপ FORM 1(a), FORM 1(b), FORM 1(c) প্রতিস্থাপিত হইবে, যথা ঃ—

FORM 1(a)

For Chemical Pesticides

(See rule 3)

APPLICATION FOR REGISTRATION OF CHEMICAL PESTICIDES

1.	**Name and address of the applicant**	**:**
2.	**Name of the pesticide**	**:**
3.	**Name and address of the manufacturer**	**:**
4.	**Common Name/ Descriptive name**	**:**
5.	**Chemical Name (IUPAC nomenclature)**	**:**
6.	**Structural formula**	**:**
7.	**Empirical formula and molecular weight**	**:**
8.	**Manufacturer's development code number(s)**	**:**

9.	**Active ingredient (certified percentage of active material)**	**:**
(a)	Physical state	:
(b)	Colour/Apperance	:
(c)	Odour	:
(d)	Refractive index	:
(e)	Melting point	:
(f)	Decomposition point	:
(g)	Viscosity	:
(h)	Boiling point	:
(i)	Vapour pressure (Figures should be given at a stated temperature preferably is the range of (20-25°C)	
(j)	Flash point	:
(k)	Specific gravity/Density (for liquids only)	:
(l)	Hydrolysis rate under stated relevant conditions	:
(m)	Surface tension	:
(n)	Stability	:
(o)	Solubility	:
(p)	Compatibility	
(q)	Photolysis	:
(r)	Absorption spectra, e. g. ultra-violet and infra red,	:
(s)	Any other relevant properties	:
(t)	Acidity/Alkalinity/pH value	:
10.	**Technical grade material**	**:**
(a)	**Source :** name and address of manufacturer and address where manufactured	:

(b)	Physical state	:
(c)	Colour	:
(e)	Acidity/Alkalinity/pH value	:
(f)	Specific gravity	:
(g)	Viscosity	:
(h)	Flash Point	:
(i)	Minimum (and maximum) active ingredient content in % w/w	:
(j)	Identity and amount of isomers, impurities and other by products together with information on their possible range expressed as % W/W	:
(k)	Storage stability (Low and High temperature storage stability).	:
11.	**Formulated product**	**:**
(1)	Identity/appearance (colour)	**:**
(2)	Odour	**:**
(3)	Type of formulation	**:**
(4)	Contents of active ingredient(s)	**:**
(5)	Content and nature (identity, if possible, of other components included in the formulation, e.g., technical grade, adjuvants and inert ingredient).	**:**
(6)	Water content/Moisture (above relevant)	**:**
(7)	Specific gravity	**:**
(8)	Viscosity	**:**
(9)	Low & High temperature storge stability (in respect to composition and physical properties related to use).	**:**
(10)	Impurities	**:**

(11)	Flammability	:
	(a) **Liquids :** Flash point	:
	(b) **Solids :** A statement must be made as to whether the product is flammable Non solid	:
(12)	Acidity	:
(13)	Alkalinity	:
(14)	P^H Value	:
(15)	Other properties may in certain cases need evaluation	:
(16)	Carrier materials	:
(17)	Wettability (for dispersible powders)	:
(18)	Persistent foam (for formulation applied in water)	:
(19)	Suspensibility (for dispersible powders and suspension concentrates)	:
(20)	Particle size	:
(21)	Wet sieve test (for dispersible powders and suspension concentrates)	:
(22)	Dry sieve test (for granules, dusts)	:
(23)	Emulsion Stability (for emulsifiable concentrate)	:
(24)	Bulk density	:
(25)	Corrosiveness (when necessary)	:
(26)	Flow ability	:
(27)	In case tablet/pellets	:
	(a) Weight	:
	(b) Thickness/height	:
	(c) Diameter	:
	(d) Colour/Appearance	:
	(e) Percentage of active ingredients and other related standard specifications	:
(28)	Known incompatibility with other products, e.g., pesticides, fertilizers	:
(29)	Application with dosage rate	:

12.	**Rate of release of active ingredient (granules, dust, etc.)**	**:**
13.	**Efficacy**	**:**
	Primary evaluation data using harmonized method and reported in a systematically presented complete dossier	
14.	**"Toxicological data of Technical Grade Active Ingredient (TGAI) and Formulated Product (FP)"**	**:**
(a)	Acute	**:**
(b)	Acute percentaneous toxicity	**:**
(c)	Acute inhalation toxicity	**:**
(d)	Acute other routes e.g., intraperitoneal	**:**
(e)	Skin irritation	**:**
(f)	Eye irritation	**:**
(g)	Short term oral administration	**:**
(h)	Short term sensitizing effects	**:**
(i)	Toxic effects of metabolites	**:**
(j)	Metabolic studies	**:**
(k)	Long term toxicity including: carcinogenicity	**:**
(l)	Neurotoxicity	**:**
(m)	Reproduction studies	**:**
(n)	Embryotoxicity, including teratogenicity	**:**
(o)	Mutagenicity	**:**
(p)	Potentiation	**:**
(q)	Direct observations, e.g., clinical cases	**:**
(r)	Health records, both from industry and agriculture	**:**
(s)	Treatment of poisoning	**:**
(t)	First aid measure	**:**
(u)	Supplementary treatment	**:**
(v)	Waiting period (last application to harvesting).	**:**
15.	**Residue studies**	**:**
(a)	Primary physical, chemical and biological data.	**:**

(b)	Identification of residue design of analytical method	:
(c)	Reliable residue data from supervised trials	:
(d)	Estimations of maximum residue	:
(e)	Data on further disappearance on storage, transport, etc.	:
(f)	Estimation of residue level in commodity on sale	:
(g)	Data on disappearance on food preparation cooking or processing	:
(h)	Prediction of potential consumer intake, actual intake studies.	:
(i)	Assessment of actual consumer intake	:
(j)	Persistence of the product	:
16.	**Prediction of Environment effects**	:
(a)	Fate and mobility studies of toxicant	:
(b)	Method of application of pesticide	:
(c)	Time of application	:
(d)	Rate of application	:
(e)	Scale of use (number of application, etc).	:
(f)	Climatic and geographical locality	:
(g)	Volatility of product	:
(h)	Water solubility	:
(i)	Octanol water partition coefficient	:
(j)	Absorption	:
(k)	Desorption	:
(l)	Degradation	:
(m)	Persistence	:
(n)	Effect on birds	:
(o)	Effect on fish	:
(p)	Effect on fish food species	:
(q)	Effect on honey bee	:
(r)	Degradation product in soil	:
(s)	Possibilities of accumulation with stable lipophillic compound	:
(t)	Effect on local aquatic species	:

(u)	Effect on soil organism	:
(v)	Disposal of used, condemend and surplus pesticides and pesticides containers	:
(w)	Proposal for labelling and direction for use as per pesticide rule 1985	:
17.	**Packaging**	:
(a)	State weight (or for liquid volume) and the sizes of package in which the products are to be marketed and for each size, the type of package, for instance i.e. 1 Kg in cans with screw plug and 50 kg in iron drums. (please note that the product must be sold only in the package size and type notified to the Plant Protection Wing, Department of Agricultural Extension and for which the label is approved.)	:
(b)	Classification during transport	:
18.	**Method of analyses**	
(a)	Methods to determine the active ingredients of the product (the accuracy of the method of determination should be stated).	:
(b)	Methods to determine the amount of isomers, impurities and other by products	:
19.	**Labelled samples for analyses**	:
(a)	Analytical reference standard 2—5g	:
(b)	Technical grade material 0.5—1.0 Kg	:
(c)	Formulated product 1 Kg/lit for each formulation	:
20.	**Registration fee:**	:
	Taka.....(Taka.....thousand) deposited in Treasury Challan No. dated (Original Treasury Challan enclosed) under Head of Account **"1**-4331-0000-2043"	:

I do hereby apply for registration of the pesticide, particulars of which are given above, and hereby certify that these particulars are, to the best of my knowledge, true and correct.

Explanation—In this Form."Active ingredient" means an ingredient capable in itself of preventing, destroying, repelling or mitigating insects, fungi, bacteria, nematodes viruses, rodents, weeds or other pests when used in the same manner and for the same purpose and those for which it is intended but is not antagonistic to the activity of any other active ingredient in the same formulation.

Date———————— ————————————

Signature of applicant

Notes

Directions for completion and submission of application. (in triplicate)

1. The application must be accompanied by :—

    a. General literature of the product including toxicological and efficacy data.

    b. Standard specification of technical product and formulation of the product.

    c. Statement of ingredients (active and inert materials to be enclosed separately in a sealed and confidential cover).

    d. Composition of formulation in details with percentage.

2. Certified true copy of the contract/agreement made between the manufacturer and the local agent authenticated by the competent agency of the country for import and marketing the product in Bangladesh.

3. (a) In case of renewal of an existing registration, the previous certificate of registration; and

    (b) A suitable sample of the pesticide sufficient for test and analysis (physical and chemical properties).

4. Treasury challan of Taka.....thousand evidencing payment in the head of account: "1-4331-0000-2043" shall be submitted.

5. Submission of application in a sealed cover marked "Confidential".

FORM 1(b)

For Biochemical Pesticide

(**See rule 3**)

APPLICATION FOR REGISTRATION OF BIOCHEMICAL PESTICIDES[1]

1.	Name and address of the applicant	**:**
2.	Name of the pesticide	**:**
3.	Name and address of the manufacturer	**:**
4.	Common Name/Descriptive Name	**:**
5.	Active Ingredient	**:**
1.	***Chemistry and other relevant particulars***	
a.	Type (Pheromone/Allomone/Kairomone/ synomone/Hormone/NPR/IGR/Botanical)[2]	**:**
b.	Natural of Synthetic	**:**
c.	Identity of Natural Source	**:**
d.	Chemical Abstract Name	:
e.	IUPAC nomenclature	:
f.	Structural formula	:
g.	Empirical formula and molecular weight	:
h.	Manufacturer's development code number(s)	:
i.	Manufacturing Process (Attach details)	:
j.	Certified percentage (Purity %) (Attach detailed method of purity identification by GC/SP techniques)	:
k.	Physical and Chemical properties	
	(i) Colour/Appearance	:
	(ii) Odour	:
	(iii) Refractive index	:
	(iv) Melting point	:

	(v) Decomposition point	:
	(iv) Viscosity	:
	(vii) Boiling point	:
	(viii) Vapour pressure (Figures should be given at a sated temperature preferably in the range of (20—25^{o}C)	:
	(ix) Flash point	:
	(x) Specific gravity/density (for liquids only)	:
(xi)	Hydrolysis rate	:
(xii)	Surface tension	:
(xiii)	Stability	:
(xiv)	Storage condition with range of temperature	:
(xv)	Solubility	:
(xvi)	Compatibility (Required only in case its use in combination with other pesticides or agrochemicals is recommended)	:
(xvii)	Photolysis	:
(xviii)	Absorption spectra, e.g., ultra-violet and infra red, etc.	:
(xix)	Any other relevant properties	:
(xx)	Acidity/Alkalinity/P^{H} value	:
II.	**Biological Properties**	
(a)	Mode of action	:
(b)	Degree of specificity	:
(c)	Target pest (s)	:
(d)	Target crop (s)	:
	Dosage rate (g/acre/year of a,i.)	:

6.	**Technical grade material**	
(a)	Source : name and address of manufacturer and address where manufactured	**:**
(b)	Physical and chemical properties	**:**
(i)	Colour	**:**
(ii)	Acidity/Alkalinity/P^H value	**:**
(iii)	Specific gravity	**:**
(iv)	Viscosity	**:**
(v)	Flash Point	**:**
(vi)	Minimum (and maximum) active ingredient content in g/Kg or g/L	**:**
(vii)	Identity and amount of isomers, impurities and other by products together with information on their possible range expressed in g/Kg or g/L	**:**
(viii)	Description of starting materials, production process and potential impurities	**:**
(ix)	Storage stability (Low and High temperature storage stability)	**:**
7.	**Formulated product :**	**:**
(1)	Type of formulation	**:**
(2)	Contents of active ingredient (s) g/Kg or g/L	**:**
(3)	Content and nature (identity if possible) of other components included in the formulation, e.g. technical grade, adjuvants and inert ingredient in g/Kg or g/L	**:**
(4)	Physical and chemical properties	**:**
(i)	Identity/appearance (colour)	**:**
(ii)	Odour	**:**

(iii)	Water content/Moisture (above relevant)	:
(iv)	Specific gravity	:
(v)	Viscosity	:
(vi)	Low & High temperature storage stability (in respect to composition and physical properties related to use).	:
(vii)	Impurities	:
(viii)	Flammability	:
(a)	Liquids : Flash point	:
(b)	Solids : A statement must be made as to whether the product is flammable	:
(ix)	Acidity	:
(x)	Alkalinity	:
(xi)	pH Value	:
(xii)	Other properties may, in certain cases, need evaluation	:
(xiii)	Carrier materials	:
(xiv)	Wettability (for dispersible powders)	:
(xv)	Persistent foam (for formulation applied in water)	:
(xvi)	Suspensibility (for dispersible powders and suspension concentrates)	:
(xvii)	Particle size (for dispersible powders)	:
(xviii)	Wet sieve test (for dispersible powders and suspension concentrates)	:
(xix)	Dry sieve test (for granules, dusts)	:
(xx)	Emulsion Stability (for emulsifiable concentrate)	
(xxi)	Bulk density	:
(xxii)	Corrosiveness (when necessary)	:
(xxiii)	Flowability (for dust)	:

(xxiv)	Following information for special formulations (tablet/pellets/microcapsule/ lures/traps) (must comply with standard specification-Annexure 1)	:
(a)	Type	:
(b)	Percentage of active ingredients and other related standard specificaitons	:
(c)	Weight	:
(d)	Thickness/height	:
(e)	Diameter/size	:
(f)	Appearance/shape	:
(g)	release rate	:
(xxv)	Known incompatibility with other products, e.g., pesticides, fertilizers	:
8.	**Toxicology Data of Technical Grade Active Ingredient (TGAI) and Formulated product (FP)**	:
(a)	Acute oral toxicity in rats and mice	:
(b)	Acute dermal toxicity	:
(c)	Acute inhalation toxicity	:
(d)	Skin irritation	:
(e)	Irritation to mucous membrane	:
(f)	Eye irritation	:
(g)	Short term (90 days) oral feeding effect only for TGAI (1 species)[3,4]	:
(h)	Short term (90 days) dermal toxicity for TGAI only (1 species)[**3,4**]	:
(i)	Metabolic studies in animals[**3,4**]	:
(j)	Cellular immune response/Immunotoxicity[**3**]	:
(k)	Reproduction studies[3,4] only for TGAI	:
(l)	Embryotoxicity, including teratogenicity[3] only for TGAI.	:

(m)	Mutagenicity[3] only for TGAI	:
(n)	Long term toxicity including carcinogenicity[4]	:
(p)	Potentiation	:
(q)	Direct observations, e.g. clinical cases	:
(r)	Health records, both from industry and agriculture.	:
(s)	Treatment of poisoning	:
(t)	First aid measure	:
(u)	Supplementary treatment	:
(v)	Waiting period (last application to harvesting) for FP	:
9.	**Rate of release of active ingredient**	:
10.	**Bio Efficacy of FP**	
I.	**Laboratory Test**	
(a)	Effectiveness of Lure/dispenser manufactured from the TGAI (Effectiveness of Lure/dispenser manufactured from the TGAI should be tested by using Wind Tunnel and should demonstrate at least 50% efficacy)	:
II.	**Field test**	:
(a)	The data on bio-efficacy based on two seasons field trials conducted following proper design and standard method under local agroclimatic conditions under the supervision of recognized national organization and presented in the form of authentic/published report.	
III.	**Application**	
(a)	Purpose of application	:
(b)	Target pest (s)	:
(c)	Target crop (s)	:

(d)	Time of application	:
(e)	Method of application	:
(f)	Rate of application (g/acre/year)	:
(g)	Limitation (s) of use	:
11.	**Residue studies**[5]	:
(a)	Chemical identity of residues	:
(b)	Nature of residues in plants, commodity and livestock	:
(c)	Reliable residue data from supervised trials	:
(d)	Estimations of maximum residue	:
(e)	Proposed Maximum Residue Limit (If expected concentration greatly exceed levels from naturally occurring substance)	:
(f)	Estimation of residue level in commodity on sale	:
(g)	Data on disappearance on food preparation cooking or processing	:
(h)	Prediction of potential consumer intake, actual intake studies.	:
(i)	Assessment of actual consumer intake	:
(j)	Persistence of the product	:
12.	**Prediction of Environmental effects**	:
(a)	Concentration of naturally occurring substance	:
(b)	Rate of use (gm/acre/year)	:
(c)	Desorption	:
(d)	Degradation	:
(e)	Persistence	:
(f)	Effect on birds	:
(g)	Effect on fish	:
(h)	Effect on fish food species	:
(i)	Effect on honey bee	:
(j)	Degradation product in soil	:

	Possibilities of accumulation with stable lipophillic compound	
(l)	Effect on local aquatic species	:
(m)	Effect on soil organism	:
(n)	Disposal of used, condemned and surplus pesticides and pesticides containers.	:
(o)	Proposal for labelling and direction for use as per pesticide rule 1985	:
13.	**Packaging**	**:**
(a)	State weight (or for liquid volume) and the sizes of package in which the products are to be marketed and for each size, the type of package, for instance i.e. 1 kg in cans with screw plug and 50 kg in iron drums. (Please note that the product must be sold only in the package size and type notified to the Plant Protection Wing, Department of Agricultural Extension and for which the label is approved.	:
(b)	Classification during transport	:
14.	**Method of analyses**	**:**
(a)	Methods to determine the active ingredient of the TG and FP (the accuracy of the method of determination should be stated)	:
(b)	Methods to determine the amount of isomers, products	:
15.	**Labelled samples for analyses**	**:**
(a)	Analytical reference standard : 10—50 g	:
(b)	Technical grade material : 50—200 g	:
(c)	Formulated product 200—500 g for each formulation	:

16.	**Registration fee**	**:**
	Taka.....) deposited in Treasury Challan No. dated (original Treasury Challan enclosed) under Head of Account "1-4331-0000-2043	:

[1] Biochemical pesticides are naturally-occurring substances or chemical synthesized by man, which must be structurally identical to a naturally occurring chemical and that must exhibit mode of action other than direct toxicity in the target pest (for example, growth regulation, mating disruption, attraction). For a synthetic chemical to be identical in chemical structure to a naturally occurring chemical, the molecular structure of the major component of the synthetic chemical must be the same as the molecular structure of the naturally occurring analog. (For details see Enclosure I).

[2] **Pheromones** are substances emitted by individuals of one species, which modify the behaviour of others within the same species. **Allomones** are chemicals emitted by one species, which modify the behaviour of a different species, to the benefit of the emitting species. **Kairomones** are chemicals emitted by one species, which modify the behaviour of a different species to the benefit of the receptor species. **Symonones** are chemicals emitted by one species, which modify the behaviour of a different species to the benefit of both the species. **Hormones** are biochemical agents that are synthesized in one part of an organism and translocated to another where they have controlling, behavioural or regulating effects. **Natural plant regulators (NPR**) are chemicals produced by plants that have inhibitory, stimulatory, or other modifying effects on the same or other species of plants. Some of these are termed "plant hormones" or "Phptohormones". **Insect growth regulators (IGR)** are chemicals that have toxic, inhibitory, stimulatory, or other modifying effects on the insect growth cycle. Botanical will include only Azadirachtin based formulation .

[3] Pheromones used in solid matrix, dispenses and having low annual use rates (less than 150 gms/acre/year) can be waived of these data.

[4] Naturally occurring short-chained lepidopteran pheromones having low anuual use rates (less than 150 gms/acre/year) can be waived of these data.

[5] All information are not mandatory; whatever data are available from published source will be acceptable.

I do hereby apply for registration of the pesticide, particulars of which are given above, and hereby certify that these particulars are to the best of my knowledge, true and correct.

Explanation.—In this Form. "Active ingredient" means an ingredient capable in itself of preventing, destroying, repelling or mitigating insects, fungi, bacteria, nematodes viruses, rodents, weeds or other pests when used in the same manner and for the same purpose and those for which it is intended but is not antagonistic to the activity of any other active ingredient in the same formulation.

Date ---------- ----------

Signature of applicant

Notes

Directions for completion and submission of application. (in triplicate)

1. The application must be accompanied by :

   General literature of the product including toxicological and efficacy date.

   Standard specification of technical Product and formulation of the product.

   Statement of ingredients (active and inert materials to be enclosed separately in a sealed and confidential cover). Composition of formulation in details with percentage.

2. Certified true copy of the contract/agreement made between the manufacturer and the local agent authenticated by the competent agency of the country for import and marketing the product in Bangladesh.

3. (a) In case of renewal of an existing registration, the previous certificate of registration

   (b) A suitable sample of the pesticide sufficient for test and analysis (physical and chemical properties).

4. Treasury challan of Take ---------- thousand evidencing Payment in the head of account : "1-4333-0000-2043" shall be submitted.

5. Submission of application in a sealed cover marked " Confidential".

FROM 1(c)

For Microbial Pesticides
(See rules 3)

APPLICATION FOR REGISTRATION OF MICROBIAL PESTICIDES[1]

1.	Name and address of the applicant	:
2.	Name of the Pesticide	:
3.	Name and address of the manufacturer	:
4.	Common Name	:
5.	Technical Grade Active Agent	
1.	**Systematics and other relevant particulars**	
a.	Type (Bacterial/Virus/Fungus/ Protozoa)	:
b.	Systematic/Scientific name	:
(i)	Genus (if applicable)	:
(ii)	Species (if applicable)	:
(iii)	Serotype (if applicable)	:
(iv)	Strain (if applicable)	:
c.	Natural occurrence/Source and Origin	:
d.	Manufacturer's development code number(s)	:
e.	Manufacturing Process (The cultures are multiplied by liquid solid fermentation. Information pertaining to user of entire mycelial mats with spores separated should be provided[2])	:
f.	Specifications (Must comply with the recommended standard available from PPW for each type of pesticide)	:
(i)	Form/Appearance	:
(ii)	Moisture content	:
(iii)	P^H	:
(iv)	Particle size	:

(v)	Suspensibility	:
(vi)	Miscibility	:
g.	Composition	:
(i)	Delta Endotoxin content [3]	:
(ii)	Beta Exotoxin content[3]	:
(iii)	Viable spore conut[3]	:
(iv)	Adjuvants	:
(v)	Human pathogens/pathogenic contaminants (Attach culture method)	:
(vi)	Other microbial contaminants (not more than 10/gm	:
(vii)	Other unintentional ingredients, their nature and identity, and quantity	:
h.	Test procedures and criteria used for identification, morphology, biochemistry, serology,immunology[3]	:
(1)	Morphology description, particle size, heat resistant spore count[3]	:
(2)	Immunology assays (Elisa/Dot Blot assay test)[3] (Appendix V1)	:
(3)	Routine test: [3] (Appendix 1 & iv)	:
(i)	Level of toxin by Housefly Bioassay method relevant conditions	:
(ii)	Potency of product by Bioassay method	:
(4)	Test methods/analysis	:
(i)	Bioassay method [3] (Appendix 1, II & IV)	:
	— LC_{50} on target larvae and potency against a reference using artificial diet or leaf disc method or in water for mosquito	:
	— Housefly Bioassay method for Beta exotoxin (for Bt) and Chemical contaminants	:

(ii)	Dual culture for antagonistic fungi/ bacteria	:
(iii)	Pathogenicity test on insect in case of entomopathogenic fungi	:
(iv)	Qualitative analysis[2]	:
	(a) CFU on selective medium	:
	(b) Test for gram negative bacterial Contaminants	:
	(c) Test for moisture content	:
(v)	Test procedure/Method of analysis[4]	:
	(a) An appropriate test procedure and criteria used for identification by Restriction Enzymes analysis test	:
	(b) Method of analysis (Standard counting method attached as appendix VII should be follow	:
	(c) Biological assays (Bioassay tests by Diet Surface Contamination method against second instar host insect should be generated for determining the LC_{50} of the formulation as against standards) (Appendix V111).	:
(5).	Viable Spore counts[3] (Appendix V).	:
(6).	Toxin content by dot blot/Elisa assay[3] (Appendix V1).	:
(7).	Technique for separation and purification of crystals[3] (Appendix 111).	:
i.	Shelf life claim	:
(a)	Data on storage stability as per shelf lime claims; and additional two month data for six months claim, three months additional data for one years claim at two different locations at ambient temperature along with meteorological data.[2.4]	:
11.	Toxicology Data[3]	:
a.	Single exposure studies	:
(i)	Oraltoxicity/Pathogenicity/Infectivity	:

(ii)	Dermaltoxicity/Pathogeicity/Infectivity	:
(iv)	Inhalation toxicity/Pathogenicity/Infectivity	:
(v)	Primary Skin irritation	:
(vi)	Irritation to mucous membrane	:
(vii)	Eye irritation	:
(viii)	Allergy/sensitization/immuno-suppression	:
111.	Bio-efficacy[3]	
	Laboratory test : LC_{50} values for each insect species under laboratory condition generated at least at two recognized national organization	:
6.	Formulated Product (FP)	:
1.	Systematics And other relevant Particulars	:
a.	Type (Bacteria/Virus/Fungus/Protozoa)	:
b.	Systematic/Scientific name	:
(i)	Genus (if applicable)	:
(ii)	Species (if applicable)	:
(iii)	Serotype (if applicable)	:
(iv)	Strain (if applicable)	:
c.	Natural occurrence	:
d.	Manufacturer`s development code number(s)	:
e.	Manufacturing Process (the cultures are multiplied by liquid solid fermentation. Information pertaining to user of entire mycelial mats with spores separated should be provided[2])	:
f.	Specifications (Must comply with the recommended standard available from PPW for each type of pesticide)	:
(i)	Form/Appearance	:
(ii)	Moisture content	:

(iii)	P^H	:
(iv)	Particle size	:
(v)	Suspensibility	:
(vi)	Miscibility	:
g.	Composition	:
(i)	Delta Endotoxin content[3]	:
(ii)	Beta Exotoxin content[3]	:
(iii)	Viable spore count[3]	:
(iv)	Adjuvants	:
(v)	Human pathogens/pathogenic contaminants (Attach culture method)	:
(vi)	Other microbial contaminants (not more than 10/gm)	:
(vii)	Other unintentional ingredients, their nature and identity, and quantity	:
h.	Test procedures and criteria used for identification, morphology, biochemistry, serology/immunology[3]	:
(1)	Morphology description. particle size, heat resistant spore count[3]	:
(2)	Immunology assays (Elisa/Dot Blot assay test)[3]	:
(3)	Routine test :[3]	:
(i)	Level of toxin by Housefly Bioassay method and relevant conditions	:
(ii)	Potency of product by Bioassay method	:
(4)	Test methods/analysis	:
(i)	Bioassay method[3]	
	- LC_{50} on target larvae and potency against a reference using artificial diet or leaf disc method or in water for mosquito	
	- Housefly Bioassay method for Beta exotoxin (for Bt) and chemical contaminants	

(ii)	Dual culture for antagonistic fungi/bacteria	:
(iii)	Pathogencity test on insect in case of entomopathogenic fungi	:
(iv)	Qualitative analysis[2]	:
	(a) CFU on selective medium	:
	(b) Test for gram negative bacterial Contaminants	:
	(c) Test for moisture content	:
(v)	Test procedure/Method of analysis[4]	:
	(a) An appropriate test procedure and criteria used for identification by Restriction Enzymes analysis test	:
	(b) Method of analysis (Standard counting method attached as appendix 1 should be followed)	:
	(c) Biological assays (Bioassay tests by Diet Surface Contamination method against second instar host insect should be generated for determining the LC_{50} of the formulation as against standards)	:
(5).	Viable Spore counts[3]	:
(6).	Toxin content by dot blot/Elisa assay[3]	:
(7).	Technique for separation and purification of crystals[3]	:
i.	Shelf life claim	:
(a)	Data on storage stability as per shelf lime claims; and additional two months data for six months claim, three months additional data for one years claim at two different locations at ambient temperature along with meteorological data.[2,4]	:
II.	Toxicology Data of Formulated product (FP) and Primary Culture	:
a.	Single exposure studies (for formulations only)	:

(i)	Oral toxicity/Pathogenicity/Infectivity	:
(ii)	Dermal toxicity/Pathogenicity/ Infectivity	:
(iii)	Intravenous toxicity/Pathogenicity/ Infectivity [2.4]	:
(iv)	Inhalation toxicity /Pathogenicity/ Infectivity	:
(v)	Primary Skin irritation	:
(vi)	Irritation to mucous membrane	:
(vii)	Primary Eye irritation	:
(viii)	Allergy/sensitization/immuno-suppression	:
(ix)	Pulmonary toxicity /Pathogenicity/ Infectivity Infectivity[2.4] (Intra-tracheal preferred) [2.4]	:
(x)	Human safety records (Effect or lack of effects) [2.4]	:
(xi)	Cell culture[2.4] (Appendix 1X)	:
b.	Eco-toxicity (for formulations only)[3]	:
(i)	Toxicity to birds	:
(ii)	Toxicity to Fish	:
(iii)	Toxicity to honey bee	:
(iv)	Toxicity to silkworm	:
c.	For Primary Culture[2.4]	:
(i)	Single dose (rats and mouse)	:
(ii)	Single dose Pulmonary intravenous	:
(iii)	Single dose intravenous	:
(iv)	Cell culture[4] (Appendix 1X)	:
(v)	Human safety records	:
d.	Environmental Safety Testing (for formulations only) [2.4]	:
(i)	Non-target vertebrates (information on infection and pathogenicity in mammals from mammalian safety testing)	:
(ii)	Birds (information on infection and pathogenicity due to single dose-oral in bobwhite, quail, Japanese quail, mallard/pigeon and chicken)	:

(iii)	Fresh water fish (information on infection and pathogenicity due to single dose-oral in rainbow trout, blue gill sunfish, Tilapia)	:
(iv)	Terrestrial invertebrates (information on mortality of honey bee and silkworm) (not required for Trichoderma and Pseudomonas)	:
(v)	Information on mortality of earthworm or organism of similar habitat and nature (not required for Trichoderma and Pseudomonas)	
III. Bio-efficacy		:
a.	Laboratory test : LC_{50} values for each insect species under laboratory condition generated at least at two recognized national organization[3]	:
b.	Field test :	:
(i)	Efficacy of field trials conducted at least in two seasons under supervision of recognized national research organizations and presented in the form of authentic/published report.	:
(ii)	Phyto-toxicity data	:
	Effect on non-target organisms (predators/ parasites) at least in two seasons	

7. Packaging and Labeling

(a)	State weight (or for liquid volume) and this sizes of package in which the products are to be marketed and for each size, the type of package, for instance i.e. 1 kg in cans with screw plug and 50 kg in iron drums. (Please note that the product must be sold only in the package size and type notified to the Plant Protection Wing. Department of Agricultural Extension and for which the label is approved.	:
(b)	Classification during transport	:

8. Methods (cite methods mentioned above or alternate authentic methods best fit)

(1)	Culture method for human pathogens[3]	:
(2)	Procedure for morphology description, particle size, heat resistant spore count[3]	:
(3)	Immunology assays (Elisa/Dot Blot assay test)[3]	:
(4)	Routine test[3];	:
(i)	Housefly Bioassay method for Level of toxin	:
(ii)	Bioassay method for Potency of product	:
(5)	Method of analysis[3]	:
(i)	Bioassay method	:
	- LC_{50} on target larvae and potency against a reference using artificial diet or leaf disc method or in water for mosquito	:
	- Housefly Bioassay method for Beta exotoxin and chemical contaminant	:
(6)	Method for Viable Spore counts	:
(7)	Dot blot/Elisa assay for toxin content[3]	:
(8)	Technique for separation and purification of crystals[3]	:
(9)	Dual Culture for antagonistic fungi/ bacteria	:
(10)	Pathogenicity test on insect in case of entomopathogenic fungi	:
(11)	Qualitative analysis[2]	:
	(a) CFU on selective medium	:
	(b) Test for gram negative bacterial contaminants	:
	(c) Test for moisture content	:

9. Labelled samples for analyses		**:**
(a)	Primary reference standard 0.5 to 1 g	**:**
(b)	Technical grade material 100 to 200 g	**:**
(c)	Formulated product 200 to 500 g or equivalent for each formulation	**:**
10.	**Registration fee :**	**:**
	Taka.....(Taka.....thousand) deposited in Treasury Challan No. dated (original Treasury Challan enclosed) under Head of Account "1-4331-0000-2043"	**:**

[1.] Microbial pesticides consist of a naturally occurring microorganism (e.g. a bacterium, fungus, virus or protozoan) as the active ingredient. In case of bacterium and fungus also mention if it is antagonistic and in case of fungus also mention if it is entomogenous.

[2.] Applicable only for antagonistic bacteria/fungi and entomogenous fungi.

[3.] For *Bacillus* spp.

[4.] Applicable for Virus (NPV, BV and GV)

I do hereby apply for registration of the pesticide, particulars of which are given above, and hereby certify that these particulars are, to the best of my knowledge, true and correct.

Explanation—In this Form. "Active agent" means an organism capable in itself of preventing, destroying, repelling or mitigating insects, fungi, bacteria, nematodes viruses, rodents, weeds or other pests when used in the same manner and for the same purpose and those for which it is intended but is not antagonistic to the activity of any other active ingredient in the same formulation.

Date_____ _____

Signature of applicant

Notes

Directions for completion and submission of application. (in triplicate)

1. The application must be accompanied by :
General literature of the product including toxicological and efficacy data.
Standard specification of technical product and formulation of the product.
Statement of ingredients (active and inert materials to be enclosed separately in a sealed and confidential cover).
Composition of formulation in details with percentage.

2. Certified true copy of the contract/agreement made between the manufacturer and the local agent authenticated by the competent agency of the country for import and marketing the product in Bangladesh.

3. (a) In case of renewal of an existing registration, the previous certificate of registration; and

(b) A suitable sample of the pesticide sufficient for test and analysis (physical and chemical properties).

4. Treasury challan of Taka.....thousand evidencing payment in the head of account : "1-4333-0000-2043" shall be submitted.

5. Submission of application in a sealed cover marked "Confidential".";

(২৫) FORM 2 এর serial no. 5 এর পরিবর্তে নিম্নরূপ serial no. 5 এবং 6 প্রতিস্থাপিত এবং FORM এর শেষে নিম্নরূপ Conditions সংযোজিত হইবে, যথা ঃ—

"5. Name and address of the manufacturer

a) Formulated product :

b) Technical grade material :

6. Name and address of the local manufacturer :

Dhaka:

The.....20 *Signature of the Registration Authority*
Seal

Conditions :

The registration of a brand of pesticide shall be effective from the date of its registration until the thirtieth day of June of the third year following the year (fiscal) of registration";

(২৬) FORM 3 এর serial no. 5 এ "registration" শব্দটির পরিবর্তে "registration (except guarantee or ingredients)" শব্দগুলি ও বন্ধনী প্রতিস্থাপিত হইবে;

(২৭) FORM 4 এর clause (b) এর পরিবর্তে নিম্নরূপ clause (b) প্রতিস্থাপিত হইবে; যথা ঃ—

"(b) Certify that except guarantee and ingredients the following change (s) from the original registration have been accepted";

(২৮) FORM 5 এর serial no. 10 এ উল্লিখিত "৪৫-কৃষিপ্রাপ্তি-অনিষ্টকারী পোকা-মাকড়, রোগ-বালাই ব্যবস্থাপনা ও বিবিধ খাতে আয়" শব্দগুলি, সংখ্যা, চিহ্নগুলি ও কমার পরিবর্তে "1-4331-0000-2043" সংখ্যাগুলি ও চিহ্নগুলি প্রতিস্থাপিত হইবে;

(২৯) FORM 6 এর—

(অ) serial no. 1 এর "Name of the pesticide (s) (each pesticide to be separately specified)" শব্দগুলি ও বন্ধনীর পরিবর্তে নিম্নরূপ শব্দগুলি, চিহ্ন ও বন্ধনীগুলি প্রতিস্থাপিত হইবে; যথা ঃ—

"Name of the pesticide(s) (each pesticide to be separately specified) :
Brand name :

Common name;"

(আ) serial no. 5 এ উল্লিখিত "৪৫-কৃষিপ্রাপ্তি-অনিষ্টকারী পোকা-মাকড়, রোগ-বালাই ব্যবস্থাপনা ও বিবিধ খাতে আয়" শব্দগুলি, সংখ্যা, চিহ্নগুলি ও কমার পরিবর্তে "1-4331-0000-2043" সংখ্যাগুলি ও চিহ্নগুলি প্রতিস্থাপিত হইবে;

(৩০) FORM 7 এর serial no. 9 এ উল্লিখিত "৪৫-কৃষিপ্রাপ্তি-অনিষ্টকারী পোকা-মাকড়, রোগ-বালাই ব্যবস্থাপনা ও বিবিধ খাতে আয়" শব্দগুলি, সংখ্যা, চিহ্নগুলি ও কমার পরিবর্তে "1-4331-0000-2043" সংখ্যাগুলি ও চিহ্নগুলি প্রতিস্থাপিত হইবে;

(৩১) FORM 8 এর serial no. 7 এ উল্লিখিত "৪৫-কৃষিপ্রাপ্তি-অনিষ্টকারী পোকা-মাকড়, রোগ-বালাই ব্যবস্থাপনা ও বিবিধ খাতে আয়" শব্দগুলি, সংখ্যা, চিহ্নগুলি ও কমার পরিবর্তে "1-4331-0000-2043" সংখ্যাগুলি ও চিহ্নগুলি প্রতিস্থাপিত হইবে;

(৩২) FORM 9 এর—

(অ) serial no. 2 এ "manufacturer/formulators" শব্দগুলি ও চিহ্নের পরিবর্তে "manufacturer"শব্দটি প্রতিস্থাপিত হইবে;

(আ) serial no. 5 এ উল্লিখিত "৪৫-কৃষিপ্রাপ্তি-অনিষ্টকারী পোকা-মাকড়, রোগ-বালাই ব্যবস্থাপনা ও বিবিধ খাতে আয়" শব্দগুলি, সংখ্যা, চিহ্নগুলি ও কমার পরিবর্তে "1-4331-0000-2043" সংখ্যাগুলি ও চিহ্নগুলি প্রতিস্থাপিত হইবে;

(৩৩) FORM 10 এর serial no. 10 এ উল্লিখিত "৪৫-কৃষিপ্রাপ্তি-অনিষ্টকারী পোকা-মাকড়, রোগ-বালাই ব্যবস্থাপনা ও বিবিধ খাতে আয়" শব্দগুলি, সংখ্যা, চিহ্নগুলি ও কমার পরিবর্তে "1-4331-0000-2043" সংখ্যাগুলি ও চিহ্নগুলি প্রতিস্থাপিত হইবে;

(৩৪) FORM 11 এর note 2 এ উল্লিখিত "৪৫-কৃষিপ্রাপ্তি-অনিষ্টকারী পোকা-মাকড়, রোগ-বালাই ব্যবস্থাপনা ও বিবিধ খাতে আয়" শব্দগুলি, সংখ্যা, চিহ্নগুলি ও কমার পরিবর্তে "1-4331-0000-2043" সংখ্যাগুলি ও চিহ্নগুলি প্রতিস্থাপিত হইবে;

(৩৫) FORM 12 এর পর নিম্নরূপ নূতন FORM 12(a) সন্নিবেশিত হইবে, যথা ঃ—

"FORM 12(a)

[See rule 12(2)]

Licence to import pesticide (s) (Technical grade material)

1. M/s.....is hereby granted licence to import the following technical grade material :—

   (a) Name of technical to the imported :

   (b) Brand name of pesticide (s) with registration No.

2. Name of manufacturer of technical grade material

3. Quantities to be imported;

4. The licence shall be in force for a period of two years from the date of issue.

Licence No.....

Date.....

Licensing Authority
Seal.

Conditions

1. The licence shall be displayed in a prominent place of the office premises.

2. The licence shall comply with the provision of the Pesticide Ordinance, 1971 ant the rules made there under for the time being in force, provided that the condition shall not apply to import any pesticide for experimental or research purposes.

3. Renewal.";

(৩৬) FORM 27 এর পর নিম্নরূপ নূতন Enclosure, Annexures এবং Appendixes সংযোজিত হইবে, যথা ঃ—

"ENCLOSURE

DEFINITIONS, SALIENT FEATURES AND CLASSIFICATION OF BIOPESTICIDES, AND THEIR DATA REQUIREMENTS FOR REGISTRATION

1.0 DEFINITION OF BIOPESTICIDE :

According to EPA, biopesticides are certain types of pesticides derived from such nutural materials as animals, plants, bacteria and certain minerals. According to FAO, biopesticides are naturally occurring substances or their synthetic analogues or genetically modified agents that are distinguished from conventional chemical pesticides by their unique mode of action, low use volume, and target species specificity.

2.0 CLASSIFICATION OF BIOPESTICIDES

Biopesticides fallinto following three major classes.

(i) Biochemical pesticides;

(ii) Microbial pesticides;

(iii) Plant-incorporated protectants (PIPs).

2.1 Biochemical pesticides

Biochemical pesticides are nuturally occurring substances that control pests by a mode of action other than direct toxicity in the target pest. A chemical must meet the following two criteria in order to be classified as a biochemical pesticide and to be subject to the data requirements for this class of compounds :

—The chemical must exhibit a mode of action other than direct toxicity in the target pest (e.g growth regulation, mating disruption, attraction). Pesticides such as **strychnine, rotenone, nicotine and pyrethrin**, which exhibit direct toxicity, are not considered biochemical pesticides; and

—A biochemical pesticides must be naturally occurring, or if the chemical is synthesized by man, then it must be structurally identical to a naturally occurring chemical. For a synthetic chemical to be identical in chemical structure to a naturally occurring chemical, the molecular structure of the major component of the synthetic chemical must be the same as the molecular structure of the naturally occurring analogy. Minor differences between the stereo chemical isomer ratios (found in the naturally occurring compound compared to the synthetic compound) will normally not rule out of a chemical being classified as a biochemical pest control agent unless an isomer is found to have significantly different toxicological properties than another isomer.

There are situations where a candidate chemical posseses many characteristics of a biological pesticides, but does not technically meet the two criteria established for defining biochemical pesesticides. The sub-technical Committee formed by PTAC should evaluate such chemicals on a case-by-case basis to determine whether, it should be classified as a biochemical pesticide or a

conventional pesticide. For example, a case-by-case evaluation would be required if the exact molecular structure of the naturally occurring compound is unknown, or if the synthetic chemical is closely related to but not identical in structure to the naturally occurring compound, or if the mode of action is different in the traget, compared to non-target organisms.

In these case-by-case situations, the criteria to be used to determine whether the chemical is a biochemical pesticide, include :

—the chemical and toxicological significance of the differences in chemical structure;

—the mode of action of the synthetic analog in the target species as compared to the mode of action of the naturally occurrning compound;

—differences in toxicity between the naturally occurring chemical and the synthetic analog.

Sub-classes of Biochemical pesticides :

Biochemical pesticides fall into four general biologically functional classes such as Semiochemicals, Hormones, Natural plant regulators and insect growth regulators, and Enzymes. Brief description of each subclass is given below:

2.1.1 Semiochemicals

Semiochemicals (SC) are chemicals emitted by plants, animals, and other organisms, and synthetic analogues of such substances that evoke a behavioural or physiological response in individuals of the same or other species. They include pheromones and allelochemicals. Pheromones are substances emitted by individuals of one species, which modify the behaviour of others within the same species. Allelochemicals are semiochemicals produced by individuals of one species that modify the behaviour of individuals of a different species. Allelochemicals include allomones, kairomones and synomones. Allomones are chemicals emitted by one species, which modify the behaviour of different species, to the benefit of the emitting species. Kairomones are chemicals emitted by one species, which modify the behaviour of a different species to the benefit of the receptor species. Synomones are chemicals emitted by one species, which modify the behaviour of a different species to the benefit of both the species.

2.1.2 Hormones

Hormones are biochemical agents that are synthesized in one part of an organism and translocated to another where they have controlling, behavioural or regulating effects.

2.1.3 Natural plant regulators and insect growth regulators

Natural plant regulators are chemicals produced by plants that have inhibitory, stimulatory, or other modifying effects on the same or other species of plants. Some of these are termed “plant homones” or “phytohormones”. Insect growth regulators are chemicals that have toxic, inhibitory, stimulator, or other modifying effects on the insect growth cycle.

2.1.4 Enzymes

Enzymes are defined as protein molecules that are the instruments for expression of gene action and that catalyze chemical reactions.

2.1.5 Botanicals

Although not covered by FAO definition, Azadirachtin, a naturally occurring component of neem, which controls a number of pests through non-toxic mechanism of action such as molting disruption, antifeedence, desiccation etc., may be considered as biochemical pesticide.

2.2 Microbial pesticides

Microbial pesticides consist of a microorganism (e.g. a becterium, fungus, virus or protozoan) as the active ingredient. The microbial pesticides include naturally occuning agents such as bacteria, fungi, viruses, and protozoa, or genetically modified such microogranisms.

2.3 Plant-incorporated protectants (PIPs)

Plant-incorporated protectants (PIPs) are pesticidal substances that plants produce from genetic material that has been added to the plant. For example, scientists can taka the gene for the Bt pesticidal protein, and introduce the gene into the plant’s own genetic material. Then the plant instead of the Bt bacterium, manufactures the substance that destroys the pest. In this case the protein and its genetic material, but not the plant itself, are to be regulated. PIPs will not be considered at present because GMOs are not still covered under national policy.

ANNEXURES

Annexure I

SPECIFICATIONS FOR PHEROMONE TRAPS AND LURES

The pheromone trap and lure should comply with the following specifications or specifications mentioned in the application as per bio-efficacy test :

1. Funnel shaped or any other standard trap : Funnel shaped trap generally used for trapping the moths of *Helicoverpa armigera, Spodoptera litura.* Earias spp. etc. or any other standard type developed for the purpose.

   (i) Colour : Any colour other Black.

   (ii) Structure : the funnel trap may have three parts (1) canopy; (2) tunnel shaped "trap base" and (3) a collection device. Or, the standard trap having the requisite parts for effective use.

   (1) Canopy Dia : 120-160mm

   Thickness : 1.0-3.0mm

   (There should be provision for fixing the canopy to the "trap base" and also the (pheromone lure)

   (2) Trap base :

   Dia of the mouth : 75-120mm

   Height of funnel : 45-190mm

   Dia of the bottom hole : 20-30mm

   Should possess a "L" or "T" shaped handle or any device by which the "trap" may be fixed to the support.

   The "Trap base" may be provided with 2 to 4 stalks for fixing the canopy to the "trap base". The canopy should be firmly rest on stalks so that the canopy is not dislodged due to wind.

   (3) Collection device : It should be made of polythene or any other suitable material. It should withstand wind, temperature and rain water.

   Should be fixed to the "trap base"in such a way that the device remains attached to the trap under field condition.

2. Sticky trap (for pink bollworm ete.)

- Corrugated DVC, plastic laminated cardboard, tin or any other suitable material that should be water-proof.
- The sticky glue should be non-driying.
- The outer surface of trap should be water-proof.
- The colour may be except black.
- There should be provision for fixing the trap for support.

3. Fly trap (for fruit/vegetable flies)

- Material construction as described in sticky/funnel trap.
- Any colour except black.
- Should withstand rainfall, heat/temperature and wind.
- Should be structured in such a way that the trap is escape proof.

Specification of Lures :

1. Lure made of sulphur free rubber/polypropylene/PVC impregnated with specific pheromone blends.
2. Field efficacy should be minimum for 15 days after application.
3. Imprgnated lures should be packed singly in individual tri-laminated pouches with 30 Ml Aluminum foil.
4. Shelf-life of Lure in original pack should be minimum 6 months at room temperature.
5. Lures should attract target insect species only, with 50% insect attractancy by pheromone/lure/dispenser by using wind tunnel method.

Other Specifications of Semiochemicals :

- For potential effects on non-target insects (predators, parasites, honeybee/ pollinators), a discussion of available information may be sufficient.
- Aquatic testing will not be required for fixed point dispensers applied over land.
- Aquatic invertebrate and fish toxicity data are required for direct application to aquatic sites.

Annexure II

SPECIFICATION FOR ANTAGONISTIC BACTERIA/FUNGI AND ENTOMOGENOUS FUNGI

1. CFU counts: *Trichoderma* 2×106 CFU/ml or g

   Entomopathogenic fungi 1×109 CFU/ml or g

2. Contaminants: *Salmonella, Shigella* or *Vibro* should be absent. Other mircrobial contaminants should not exceed 1×10^4 count/ml or g.

3. Method of analysis:

   (a) CFU counts by serial dilution and examination under phase contrast microscope.

   (b) Plating for contaminants on specific media.

   (c) Antagoxistic capability on target microbe by bioassay on plates; or

   (d) Entomopathogenic capability on target insect by bioassays.

4. An undertaking should be submitted that the strain is indigenous, naturally occurring not exotic and not genetically modified.

Annexure III

SPECIFICATIONS FOR BACULOVIRUS

1. Viral Unit : NPV-1×10^9 POB/ml or gm

   1×10^8 POB/ml or gm

   GV-5×10^9 Capsules/ml or g

   (POB-Polyhedral occlusion body)

2. Contaminations : *Salmonella, Shigella* or *Vibro* should be absent. Other microbial contaminamts should not exceed 1×10^4 count/ml or g.

3. Identification of *Baculovirus* by restriction enzyme analysis and Southern biot.

4. An undertaking should be submitted that the strain is indigenous, naturally occurring and not exotic and not genetically modified.

5. Method of analysis :

Counting for POB/Capsule as per Appendix-1

Viral Unit : NPV-1×10^9 POB/ml or gm

1×10^8 POB/ml or gm

GV-5×10^9 Capsules/ml or g

Note :

1. In case of NPVS, POB count should be taken with haemocytometer and phase contrast microscope. In case of GVs shallow depth (0.01mm) counting chamber under dark field illumination will be used.

2. Biological assay : Results of bio-efficacy tests by diet surface contamination method against second instars target should be generated for determining the LC_{50} of the formulation as against Standards as per enclosed procedure (Appendix II).

3. Plating for contaminants on specific media.

APPENDIXES

Appendix I

BIOASSAY METHOD FOR BACILLUS

The following procedure (or any other authentic alternate procedure) should be carried out :

(i) Test insects: 2-3 instars larvae of target insects

(ii) Procedure :

1 gm of Bacillus preparation is mixed with 40 ml. of distilled water and blended at 12000 rpm for one minute. Dilution with depend upon the nature of test sample or standard preparation. 10 gm. of artificial diet/9.5 ml tap water and 0.5 ml of diluted sample are mixed thoroughly. The diet/tap water incorporated sample food, the leaf discs of uniform size are dipped for 30 seconds in different dilutions of *Bacillus* and dried under fan. Thirty Larvae (10 larvae × 3 dishes) are used for each dilution of the sample or standard preparation. The larvae are incubated for 1-3 days test period. Mortalities recorded in 3-5 dilutions are recorded and plotted on log-probability paper. If more then 3 out of control larvae die, the results are discarded.

(iii) Calculation of results :

The potency of the sample is calculated by the following formula :

Potency of sample = LC 50 of standard (mg/ml) × IU/mg of standard LC 50 of sample (mg/ml)

(iv) Each registrant should prepare a "self Reference" and should deposit it with the Registering authority. Each self-reference with be expressed as IU/mg using International Standard.

Appendix II

Blot assay of *Bacillus thuringiensis* (B.t.) toxin Protein as alternate of Bioassay (or Fallow any other authentic alternate method)

(1) B.T grown till sporulation in shake flask or in fermenter vessel and let the cells lyse and release spore/crystals into the medium.

(2) Cells are hervested by centrifugation at 10k for 15 minutes.

(3) Wash the pellet with 1M NaCl to remove the B.T. assciated senie, metallo proteases and washed twice with sterile distilled water.

(4) Pellet re-suspended in 50 MM NaOH to solublize the toxin protein for 2 hours as R.T. with slow shaking and centrifuged again at 10K for 15 Minutes.

(5) Supernatant was adjusted to P^H 8.0 with the HCI P^H 8.0.

(6) Protein contents estimated by Lowry's protocol.

(7) To fold serial dilutions of test protein were made in PBS and known amount at protein applying on NCP using S&S or Biorad Dot Blot manifold apparatus and applying water vacuum for 30 minutes.

(8) NCP was carefully removed from Dot Blot set and soaked in excess of 3% Skin milk in PBS for blocking the remaining acetic sites on NCP for 2-3 hours at R.T/O/N at 4°C

(9) Wash the NCP with excess PBS with 0.01% Tween 20=3-4 times and then finally with PBS.

(10) Polyclonal antiserum raised against total crystal protein was suitably diluted in PBS and added to the "seal a meal" containing NCP and incubated for 1-2 hours with shaking.

(11) Remove the NCP from the bag and wash several times (as in step 9)

(12) Anti-rabbit antibodies conjugated with HRPO/Alkaline Phosphate was diluted as per the suppliers instruction and incubated NCP (as in step 10)

(13) Wash as in step 11

(14) For HRPO

(a) Diaminobenzeden (4mg/10ml PBS/4-Chloro-1-Napthol (4ma/10ml 20% Alcohol) were dissolved and 10 ml of 30% of H202 per 10 ul substrate solution was added and color reaction developed in dark for 5-10 mins (DAB gives brick red colour. 40N gives blue colour).

(b) For Alkaline Phosphates Buffer:

Alkaline Phosphates Buffer:

1M Tris p^H 8.8	-10 ml/
4M NaCl	-2.5ml/make up 100 ml
1M $MgCl_2$	-2-0.5ml/

For 10 ml of above buffer add NBT-66 µl and BCIP-33 µl and develop and colour reaction

(15) Stop the reaction by removing the substrate and washing with PBS

(16) Keep on filter paper and dry.

DEFFERENT PROTEIN CONCERTRATION

10µg 5µg 2.5µg 512.5ng 256.25ng 128ng 64ng 32ng 16ng 8ng 4ng

D0
i
f 0
f
e o
r
e o
n
t 0
s
a o
m
p o
l
e

Determination of cell dry weight

- Take a known volume of Bacterial culture spin down at 4R for min.
- Wash the Pellet in minimal distilled water.
- Transfer to a pre weighed container.
- Incubate at 80 C for 16-18 hours till become dry and weight becomes constant.

PURIFICATION OF CRYSTALS BY GELATIN METHOD (OR FOLLOW ANY AUTHENTIC METHOD)

- Centrifuge the sporulated material and wash pallet twice with 1M NaCl.
- Add 200 ml. of 0.5% Gelatin, stir and remove all forth completely.
- Dilute with sterile water and centrifuge.
- Take debris and stir with 20 ml. of 1.5 M sucrose Further add 50 ml of 1.5 Ml sucrose.
- Stir and centrifuge at 3000RPM for 2 hours.
- Remove supernatant and purified crystals are harvested.

Appendix IV

BETA-EXOTOXIN DETERMINATION BY HOUSE FLY BIOASSAY METHOD (OR FOLLOW ANY OTHER AUTHENTIC ALTERNATE METHOD).

Fly Assay Diet		
condition		Laboratory
Agar-	16g	Temp. 25 C +2C
Milk powder	100g	R.H. +70%
Yeast	100g	Test Insect -2 days
	old Hot	
Methyl Paraben -2.1g		fly larvae.
Water-	1000 ml	No. of replications=2

Procedure **:**

i) 1 g sample thoroughly mixed with 9 ml. of sterile saline.

This solution is heat treated at 65°C (water bath) for 45 minutes and incubate at rotary shaker for 2hrs. at room temp.

ii) Then centrifuge this sample at 12,000 RPM for 10 minutes.

iii) This suspension is serially diluted (1:10) to 10 dilutions.

iv) Liquid diet 200g for each replicate is placed in trays/beakers.

v) 5 ml. of heat-treated culture supernatant (10^{-6}) is poured on diet.

Let it solidify at room temp. For control, use 5 ml. of sterile water.

vi) 2 days old House fly larvae (50) in each replicate i.e. two replicate each for sample and control & cover with wire mesh/clot.

vii) Incubate the trays at 25°C + 2°C till emergence.

viii) After 24 hrs. just put 5 g wheat bran in each tray on the top (On 8^{th} to 10^{th} day). On adult emergence freeze the trays for 2 hrs. to count the adults and% mortality may be calculated as **:—**

Mortality + (100-Number of Normal Adults)

Appendix V

DETERMINATION OF HEAT RESISTANT VIABLE SPORE COUNT OF *BACILLUS* SPP. (OR FOLLOW ANY OTHER AUTHENTIC ALTERNATE METHOD)

Materials required :

i) Beaker/flasks
ii) Sterile Water
iii) Test Tubes
iv) Micropipettes
v) Water bath
vi) Petri plates
vi) Nutrient Agar
vii) P^H meter

Method :

i) Weigh 1g or 1ml of the Bt formulation.

ii) Transfer it in a sterile 250 ml corneal flask with 100 ml sterile water.

iii) Mix it gently to form a uniform suspension.

iv) Heat this material in a preheated water bath at 80° C for 15 minutes ensure that the vegetable cells of BT and all other microorganisms are killed except the Bt spores .

v) Add 9 ml of sterile water in each in 10 sterile test tubes number from 1 to 10.

vi) Add 1 ml of heat-treated Bt suspension to test tube no. 1

vii) Shake well and transfer 1ml of the suspension from test tube 1 to be labeled 2.

viii) Repeat the procedure till the last test tube to give the dilution of 1/100, 1/1000, 1/10,000 i.e. 10^1, 10^2, 10^3 .....10^{10} and the corresponding dilute factor ins 10, 100, 1000 .....respectively.

ix) Dispense 50ml of dilute sample suspension from 5^{th} to 10^{th} dilute in triplicate in the plates with 15—20 ml. Nutrient Agar medium.

Nutrient Agar plates should be prepared the previous day and must observed for any possible contamination.

x) Spread the sample suspension on the agar medium thoroughly with glass spreader.

xi) Incubate the plates at 30°C for 24 hours and count the number of colonies.

Calculation :

No. of viable spores/gm or ml. = $N \times D \times 2 \times 10^3$

N – no. of colonies in plate (Average of three plates)

D – Dilution factor

Appendix VI

QUANTIFICATION OF BT ENDOTOXIN USING ELISA TECHNIQUE (OR FOLLOW ANY OTHER AUTHENTIC ALTERNATE METHOD):

1. Material required :

Micropipette 1000 µl, 20 µl, 20 µl.

Microtips

Microtitre Plates

Measuring Cylinder

Electronic Balance

Beaker/Flask

Petridish

Tissue paper or Blotting Paper

Primary antisera (Antibody raised in rabbit against Bacillus Spp.

antigen (crystal proteins)
Secondary antibody (Goat-anti rabbit 1gG-aP conjugate)
ELISA Reader with 405 nm filter.
P^H meter

2. Reagents :

Phosphate Buffered saline (PBS), P^H 7.495×conc.) :

NaCl	40.0g
K_2HPO_4	1.4g
Na_2 $HPO_4 12$ H_2O	14.5g
KCL	1.0g

Make up to 1 liter and stored at 4°C

Make it 100 mM, take 200 ml of PBS and make 1 liter with water.

Blocking solution:

Skimmed Milk Powder 3% in PBS

PBST

PBS (5×) 200ml

10% Tween 20 5ml

Make up to 1 Litre.

Coating Buffer :

NaCo	1.59g
NaHCo	2.93g

Make up to 1 litre

Substrate buffer for Alkaline Phosphatase : (Prepare freshly)

Diethanolamine	97ml
Water	800ml

Adjust the P^H to 9.8 with HCL

Make up to 1 Litre.

Substrate for Alkaline Phosphatase

NitroPhenyL Phosphate (NPP) 0.5mg/ml in substrate buffer.

3. Method

Preparation of antigens :

i) Weigh 1mg of biopesticides and add 1ml of 100 mM Sodium Carbonate (P^H10)

ii) Incubate for 2 hrs at 37°C at 100 rph in and incubator shaker.

iii) Make two fold dilution of the test protein and the pure toxin protein separately in different rows.

Two fold dilution making:

i) Add 100 µl of 1000 mM sodium carbonate buffer in Columns 4 to 10 microtitre plate.

ii) Add 200 µl (containing 16 µg) of the pure crystal toxin in column 3.

iii) Add 100 µl of aliquots from column 3 to column 4 and mix well by gent pipetting.

iv) Similarly do from 4 to 5, k to 6 ..... 9 to 10.

v) Discard 100 µl from 10.

vi) Coat the test antigens in sodium carbonate buffer and incubate the plate overnight at 4°C.

vii) Use 2 to 3 replications for each sample.

viii) Record the sample data in the record chart.

ALLOW THE ELISA PLATE TO DRY AT ANY STAGE

Remove the solutions in the ELISA Plate by inverting it and gentle. tapping on a tissue paper of blotting paper.

Wash the microtitre ELISA Plate once with 150µl of PBS.

Add the Blocking solution to each well till the brim.

Incubate the plate at 37°C for 1 hr.

Add 100 µl of Primary antibody (1:1000) dilution in PBS to each well.

Incubate the plate at 37°C for 1 hr.

Wash the microtitre ELISA plate twice with PBS:

Add 100 µl of Secondary antibody conjugate (1:2000) dilution in PBS to each well.

(Secondary Antibody is Goat-anti Rabbit 1gG-AP conjugate)

Incubate the plate at 37°C for 1 hr.

Wash the microtitre ELISA plate thrice with PBST.

Wash the microtitre ELISA plate once with the substrate buffer.

Add 100 µl of substrate solution to each well.

Incubate the plate for 15 to 30 minutes at room temperature until colour develops.

Read the absorbance in an ELISA reader at 405 nm.

Using the standard curve determine the concentration of the Bt toxin unknown test samples.

Appendix VII

COUNTINC NPV (POB) USING IMPROVED NEUBAUER HAEMOCYTO-METER CR COUNTIN CHAMBER (OR FOLLOW ANY OTHER AUTHENTIC ALTERNATE METHOD)

A haemocytometer is an essential tool used for estimating the number of microorganisms in a capsule. The Improved neubauer haemocytometer comprised a thick glass slide with a shallow depression in the Central section divided into two halves. On each side, the base of the depression has a fine ruled grid of squares, which is visible under a microscope. The dimensions of this grid are defined. With a thickened cover slip placed over the depression a chamber is created of fixed depth. A small volume of test suspension is introduced to both halves of the slide chamber from a pipette and 2—5 minutes allowed for particles to sediment to the chamber floor.

Either dark field or phase contrast microscopy is used to identify and count polyhedra (POB) with the counting chamber under the microscope. The number of Polyhedara/capsules in a given number of grid squares can be counted. Each

count consists of a tally of the number of polyhedra completely contained within a square plus the number of touching the left and upper sides. Polyhedara touching the bottom and right sides are not counted. Since both depth of the chamber and the grid dimensions are known. It is then a straightforward calculation to determine the number of particles per ml of test sunpension.

Number of polyhedra (POB) perml/gm = D × ×

N × ×

Where

D = Dilution factor

X = total number of polyhedra counted

N = Number of squares counted

K = Volume above one small square in cm

Area of each small square is 1/400 mm = 0.0025 mm. Depth of chamber is 0.1 mm. Volume of liquid above a single small square is 0.0025mm × 0.1m = 0.00025mm. To convert to cm^3 multiply by 1/1000 to get a volume of 2.5 × 10 cm above 1 small square.

Example

Suppose in a sample diluted by a factor of 1000 we count 535 polyhedra in 160 small square then:

D= 1000

X= 535

N= 160

K= 2.5×10^{-7} cm^3

Thus POB count= $\frac{1000\times535}{160\times2.5\times10^{-7}}$ $\frac{5.35\times10^{3}}{4\times10^{5}}$ = 1.33×10 Polyhedra/ml undiluted sample

Usually this procedure is repeated 3 times and average taken to get a more accurate estimate.

Appendix VIII

PROCEDURE FOR ESTIMATION OF LC_{50} OF NPV BY THE STANDARD DIET SURFACE CONTAMINATION METHOD (OR FOLLOW ANY OTHER AUTHENTIC ALTERNATE METHOD) :

i) Diet to be used: The standard chickpea-based diet without formaline.

ii) Bioassay bottles: 5ml vials with a diameter of 18 mm (255 mm surface area)

iii) Doses of NPV to be tested:

	POB/ml	POB/mm^2
(10 microlitres to be dispensed for each vial)	a) 5×10^4	19.6
	b) 1×10^4	3.9
	c) 2×10^3	0.78
	d) 0.8×10^2	0.16
	e) 0.16×10^2	0.03
	f) 1.6×10^4	0.006

iv) Method of dosing : Dispense 10 Microlitre aliquots into each vial and spread uniformly over the entire diet surface using a polished rounded lip of a 4 mm glass rod and allow to dry of under hood for 10 min.

v) No. of Larvae/dose: 50 (maintain 50 larvae without virus inoculation for control)

vi) Stage of larvae: 2nd instar larvae (preferably 4 days old)

Release one larva/vial and plug mouth with sterile absorbent cotton. Incubate at 25+1°C for 7 days.

vii) Record mortality in different doses on the 7th day.

viii) Apply Abbott's correction.

ix) Subject the dose-morality response to probit analysis using a statistical soft ware in a computer.

x) Express LC_{50} as POB/mm^2 of diet surface.

Expected standards for NPV for 2nd instars larvae

Species	LC_{50} POB/mm^2
1. Helicoverpa armigera	0.5
2. Spodoptera litura	20.0

Appendix IX

TOXICITY/PATHOGENICITY STUDY BY CELL CULTURE METHOD (IN VITRO) (OR FOLLOW ANY OTHER AUTHENTIC ALTERNATE METHOD) (APPLICATION FOR VIRUS BASED BIOPESTICIDES)

I.0 Cell Culture

a. Rationale:

Viral agents may have toxic potential and or may be infectious to mammalian cells. The purpose of the following test is to assess the capability of viral pest control agent for infection and toxicity. The applicant should submit information and/or data addressing the above two aspects. If the results indicate toxicity or pathogenicity then data on additional cell line from other species shall be required.

B. Protocols:

(1) Substance to be tested:

The most infective forma of the viruses should be used for the test. The virus titer should be tittered using standard method and should be devoid of defective viral particles. For virus titration, the most susceptible host system should be used.

(2) Methods of Virus Preparation:

The process of virus manufacture should be noted with reference to cell culture method, operation, medium used and stock virus used to generate the virus product.

(3) Cell Culture:

For testing the toxicity and infectivity of the virus product an established human cell line (such as WI 38) or any other primate continuous cell line such as monkey CV-1 should be used. The source, genetic stability and the passage number of each call lines used should be provided. Sub-confluent culture (containing approximately 200 cells on 25 cm sq. dish) of each cell lines should be used for the test.

2.1 Toxicity Evaluation :

Approximately 200 cells on 25 cms sq dish are plated in petri dishes in suitable medium. Twenty-four hours after planting, virus based biopesticides (about 10 POB/MI) are introduced into the plate, after 1 hr, colonies (an aggregation of minimum 25 cells) were counted, stained and morphological evolutions are done for Cytopathic Effects (CPE) Result from the cultures of minimum of 6 plates along with the concurrent three controls to be provided.

2.2 Infectivity Evaluation

Approximately 2× 10 cells on 25cms sq dish in the media were exposed to virus-based biopesticides at 1× 10 POB/ml in petri plates. Cultures were quantitatively assayed for the virus concentration and infectivity at 1,2,5,7, 14 and 21 days if required after inoculation. Results of the cultures from 6 plates along with three concurrent controls to be provided.

2.3 Test Evaluation

Both toxicity and infectivity studies should be compared with proper control experiments. Inactivated virus of same dose can be used as negative control and permissive cell line or host organism as positive control. The results of culture from 3 plates each from negative and positive controls should be presented and reported. Any cytopathic effect and/or viral infection observed in tissue culture should be described.

The following definitions apply to this protocol :

Cytopathic Effects (CPE)

Cytopathic effects (CPE) are any host cell damage or injury resulting from infection of the cell by a virus. These effects can be morphological or biochemical, and include but are not limited to cell growth, attachment morphology, nucleus size and shape, and cellular processes such as macromolecular synthesis.

The results of CPE can be classified as follows :

(A) 1+ = Suggestive of virus-included morphologic changes.

(B) 2+ = definitive morphologic changes.

(C) 3+ = more than 50 percents cell degeneration.

(D) 4+ = complete cell destruction.

Most Infectious Form (MIF)

Most infectious form (MIF) is the form or preparation of virus that gives optimal infection in the susceptible cell culture or organism. For occluded viruses (e.g. baculoviruses, cytoplasmic polyhedroses viruses, entomopox viruses) the IMF for cell culture or injection into an organism is extracellular virus found in cell culture medium or in infectious hemolymph. The IMF for susceptible insect host for infection by natural routes (feeding) is the viral occlusion body.

Viral Infectivity :

Viral infectivity is the ability of viral genes to become established in a host cell genome, or the ability of viral genes to be expressed in a host cell (resulting in the production of virus-encoded nucleic acids). The results of the viral infectivity should be reported as titration value of virus during entire period of incubation time.

Viral Toxicity :

Viral toxicity is the ability of a virus to inflict injury or damage to a host cell. where infection by, and/or replication of the virus are not necessarily required. Toxicity can also be the ability of non-viral components or a preparation to inflict injury or damage to a host cell.

Report Preparation :

The report should contain :

1. Cell line and source
2. Cell culture method
3. Virus genotype and host
4. CTE-Data from positive and negative control and treatment group

(results of individual plates to be provide on infectivity and toxicity)"।

রাষ্ট্রপতির আদেশক্রমে

সি.কিউ.কে. মুসতাক আহমদ
সচিব।

মোঃ মজিবুর রহমান (যুগ্ম-সচিব), উপ-পরিচালক (অতিরিক্ত দায়িত্ব), বাংলাদেশ সরকারি মুদ্রণালয়, ঢাকা কর্তৃক মুদ্রিত।
মোঃ মজিবুর রহমান (যুগ্ম-সচিব), উপ-পরিচালক, বাংলাদেশ ফরম ও প্রকাশনা অফিস,
তেজগাঁও, ঢাকা কর্তৃক প্রকাশিত। web site: www.bgpress.gov.bd